Optimizing IVF results

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Cover illustration:

A scarab beetle carrying a 2PN fertilized oocyte in place of the sun disc. The Ancient Egyptians believed that the scarab was a symbol of spontaneous creation; representing the God Khepri making the sun rise. The hieroglyphic inscription added to the figure is the instructions of Ptahhotep (Sixth Dynasty: 2300-2150 BC). The translation:

> "No limit may be set to art, neither is there
> any craftsman that is fully master of his craft."

Optimizing IVF results

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Abbreviations

IVF-ET	In vitro fertilization and embryo transfer
ART	Assisted Reproduction Techniques
ICSI	Intracytoplasmic sperm injection
CC	Clomiphene Citrate
hMG	Human menopausal gonadotropins
GnRH	Gonadotropin releasing hormone
LH	Luetinizing hormone
FSH	Follicle stimulation hormone
r-hFSH	Recombinant human follicle stimulating hormone
US	Ultrasound
MESA	Microsurgical epididymal sperm aspiration
PESA	Percutaneous epididymal sperm aspiration
CAVD	Congenital absent vas deferens
TESA	Testicular sperm aspiration
KCl	Potassium chloride
eSET	Elective single embryo transfer

Chapter 1

General Introduction

History

The advent of human in vitro fertilization and embryo transfer (IVF-ET) is a breakthrough in the field of infertility. The first IVF baby was born in 1978 after ten long years of hard effort by two pioneers, Robert Edwards and Patrick Steptoe (1,2). The technique of IVF-ET entails retrieving the oocytes, fertilizing them in vitro, and then transferring the resulting embryos into the uterine cavity. The idea seems very simple, however, it is a series of delicate and complicated procedures. Robert Edwards spent many years conducting research in England and the United States, trying to obtain human oocytes to fertilize them in vitro. He obtained pieces of ovarian tissues, taken during laparotomies for ovarian wedge resection in polycystic ovaries, and tried to mature them in vitro (3, 4). The first culture of human oocytes in vitro from ovarian tissue removed at laparotomy is ascribed to Gregory Pincus about 25 years before the same experiment was tried by Edwards (3). After many years of research the first observation of a human oocyte fertilized in vitro was reported in "Nature" in 1969 (5). Then, Edwards and Steptoe were ready to begin clinical IVF. Human oocytes were successfully fertilized and grown in vitro to become cleaved embryos, however no pregnancy occurred for the first seven years. These years were characterized by criticism from various groups in society including religious people, theologians, and most of all, unfortunately, scientists and medical professionals. However, these waves of criticism stimulated Edwards and Steptoe and they continued research in every area. It was then that they realized that the failure of embryos to implant was due to luteal phase disruption (3). They were using primolut depot for luteal phase support, which unfortunately has a leuteolytic effect. Consequently, natural cycle IVF was used and the first IVF pregnancy was achieved. This event was the culmination of many years of research that had been simultaneously carried out in different places, mainly, England, Australia, and the United States (6). When the first IVF baby was born, Patrick Steptoe said " This is the first time

we've solved all the problems at once. We are at the end of the beginning-not the beginning of the end" (7). Ever since, human IVF has spread throughout the world and medical science has been getting deeper into in-vitro conception. Since the beginning of IVF, a steady stream of discoveries and progress in technology has resulted in the expansion of the indications to be treatable by IVF. Different techniques developed and the term "Assisted Reproduction Techniques" (ART) was introduced.

Results of IVF

IVF results have improved gradually and significantly over the years. The pregnancy rates differ from country to country and from one clinic to another in the same country. Tables 1 - 3 show the results of IVF and ART in the United Kingdom, Europe, and the USA in different years (8-17). The improvement in the overall success rates of assisted reproduction is attributed to several factors including the increased level of expertise, the improvement in controlled ovarian stimulation protocols after the introduction of gonadotropin releasing hormone agonist analogues, the improvement of tissue culture media, the simplification of ovum pick-up after the development of transvaginal US technique, the remarkable improvement in fertilization rates after the introduction of intracytoplasmic sperm injection (ICSI), the increased awareness and experience in the embryo transfer technique, and the improvement in luteal phase support. The following are more details of some factors that improved the IVF results:

Ovarian stimulation protocols:

The first IVF pregnancy was conceived in a natural cycle (1). However, to increase the chances of pregnancy, superovulation was introduced to induce the growth of multiple follicles. Until the late 1980s, the most common ovulation induction drugs were clomiphene

citrate (CC) and human menopausal gonadotropins (hMG) (6). The results of CC/hMG stimulation protocols demonstrated its efficacy with an overall clinical pregnancy rate per transfer of 25% (18, 19). However, a premature LH surge was a major disadvantage that necessitated the cancellation of approximately 15% of all cycles (20). Gonadotropin releasing hormone agonists (GnRH agonists) were introduced as a means of pituitary down regulation to prevent premature LH surges (21). Since their introduction, GnRH agonists have become the gold standard for ovulation induction and the pregnancy rates have increased significantly (22, 23). Two large, well-controlled studies demonstrated that the take home baby rate was doubled when GnRH agonist was used instead of the CC/hMG protocol (24, 25). Very recently, another way of prevention of premature LH surges was introduced through the use of GnRH antagonist. GnRH antagonist is a competitive inhibitor to GnRH that leads to an immediate decrease in LH and FSH secretion (26). In a recent multicenter study, the antagonist in comparison with the agonist resulted in a significantly shorter period of stimulation, a reduction in the amount of FSH consumed, a decrease in the estradiol level and about 1.3 oocytes less per pick-up (27). The clinical pregnancy rate per attempt was 32.8% in the antagonist and 37.8% in the agonist protocol with no statistically significant difference (27). A recent meta-analysis showed that there is a small, but significant difference in the pregnancy rate in favour of the agonist. However, there was no significant difference in the incidence of ovarian hyperstimulation syndrome (28). More research is needed in order to fine-tune the use of the antagonist such as using it in a more flexible way on the starting day and giving a weight adjusted dose.

Another recent development in ovulation induction drugs is the use of recombinant technology. The clinical efficacy of r-hFSH was demonstrated in IVF when multiple follicular growth, fertilization, and pregnancies were achieved (29, 30). In a prospective, multicenter study r-hFSH was compared with urinary hFSH in an IVF program. In patients

receiving r-hFSH, a significantly higher number of oocytes were retrieved with a lower total dose of FSH over a shorter period of stimulation compared with urinary hFSH. There was no significant difference in the implantation rates or the pregnancy rates, but more embryos were cryopreserved in the r-hFSH group (31). However, in a prospective randomized study comparing hMG with rFSH, when only first attempt IVF/ICSI cycles were considered, the live birth and implantation rates were significantly higher in the hMG group (32). In a meta-analysis comparing r-FSH and urinary FSH it was concluded that r-FSH was associated with a better pregnancy rate per started cycle (33). However, when this meta-analysis was updated (34) by adding five more recent trials, there was no difference (OR 1.06, 95% CI 0.94, 1.20). It is clearly demonstrated from the above that the efficacy of urinary and recombinant gonadotropins is the same, however, the cost-effectiveness will be the determining factor in choosing one over the other.

Simplification and accuracy of ovum pick-up techniques

One of the main reasons for improving IVF results is that the ovum pick-up technique became simple and accurate. In the beginning of IVF, ovum pick-up was only done through abdominal laparoscopy under general anaesthesia. A good view of the pelvic organs is essential, particularly the ovaries. For most IVF patients, due to the presence of pelvic adhesions, the procedure was not a straightforward one and could be lengthy due to adhesions covering the ovaries, endometriosis, bleeding, and difficulty in recovering oocytes.

Ultrasound-guided oocyte recovery has revolutionized the way IVF is practiced. It made IVF possible without general anaesthesia and as an outpatient procedure. The technology continuously improved, particularly in real-time scanning, leading to successful oocyte recovery through trans-abdominal ultrasound guidance (35). With further improvement in the technology, transvaginal ultrasonic-guided oocyte recovery became possible (36). Currently,

oocyte recovery is done almost universally through the transvaginal US-guided route, since it is the easiest, and the most accurate method.

Improvement in fertilization

The development of ICSI was a breakthrough in the field of assisted reproduction (37). It simply made fertilization possible in many cases that used to result in total failure of fertilization using conventional IVF and cases that were not acceptable in the IVF program in the first place. The technique of ICSI entails the injection of one spermatozoon inside the ooplasm. ICSI has been widely and successfully used to achieve fertilization in cases of oligoasthenozoospermia (38-41). It has also been successful in achieving fertilization and pregnancies using epididymal and testicular sperm in cases of obstructive and non-obstructive azoospermia (42-49). The use of ICSI in suspected male factor infertility or borderline semen (40, 50) and unexplained infertility (51) was also recommended. It significantly improved the fertilization rate and avoided the occurrence of total failure of fertilization in these cases (52). Naturally, ICSI is the first line of treatment in cases with previous failure of fertilization using conventional IVF (41). ICSI has also proven to be of value to patients with male immunological infertility (53) and with acrosomeless spermatozoa (54-56). Even in cases of totally immotile spermatozoa, although the fertilization rate is low, pregnancy and birth have been reported (57-59). Another use of ICSI is to fertilize cryothawed oocytes (60,61) and in-vitro matured oocytes (62-64). It has even been suggested that ICSI might completely replace conventional IVF. However, data from prospective randomized studies showed no significant benefit from ICSI over conventional IVF in cases with normal semen (65). A multi-center randomized controlled trial comparing clinical outcome after ICSI or conventional IVF in couples with non-male factor infertility was recently performed (66). It was concluded that ICSI offers no advantage over IVF in

terms of clinical outcome in cases of non-male factor infertility (66). From both safety and scientific viewpoint, it is concluded that ICSI should only be used if success at conventional IVF is regarded unlikely (67). The technique of ICSI has significantly improved fertilization. Cases of severe oligoathenospermia can achieve fertilization rates from 60 to 76% (41, 68, 69). Moreover, the results of ICSI using either ejaculated, epididymal or testicular spermatozoa did not differ significantly (70). The results of a Cochran systematic review showed that there is evidence that fertilization rates are significantly better with ICSI than IVF in couples with borderline semen (71). When the semen parameters are normal there is insufficient evidence that ICSI is more effective than IVF when the fertilization rate is calculated per retrieved oocyte. However there is small but statistically significant improvement in fertilization rate per inseminated oocyte (71).

Improvement of tissue culture media

Basically, tissue culture media is composed of a balanced salt solution with added carbohydrates and amino acids. Extensive scientific research has been done to develop tissue culture media that will successfully support the development of viable human embryos. Many controlled studies have demonstrated that fertilization and cleavage can successfully occur in a variety of simple and complex media (72). Not only the composition of the media is important but also rigorous quality control is essential. Each batch of culture media must be free of endotoxins, microorganisms, and low in ion content. It should be checked for embryo toxicity before use (73).

Synopsis

The development of IVF is a breakthrough in the field of infertility. After many years of research, the first IVF baby was born in 1978. The results of IVF have improved gradually but significantly over the years. The improvement in the results of IVF is due to several factors including the increased level of expertise, the improvement of controlled ovarian

stimulation protocols, the improvement of tissue culture media, the simplification of ovum pick-up through the use of transvaginal ultrasonic route, the significant improvement in fertilization due to the development of ICSI, the increased awareness and experience in the embryo transfer technique, and improving the luteal phase support.

Table 1. Live birth rates for IVF and micromanipulation in the United Kingdom. *

Reporting period	IVF		Micromanipulation	
	Number of Treatment Cycles	Live Birth Rate per Treatment Cycle (%)	Number of Treatment cycles	Live Birth Rates per Treatment Cycle (%)
91/92	10434	14.0	80	3.8
92/93	19309	13.1	244	5.7
93/94	21726	14.3	298	9.3
94/95	24193	14.3	1685	15.7
95/96	25781	14.3	4651	20.2
96/97	26865	15.5	6652	21.6
97/98	24889	14.9	9295	20.7
98/99	23254	16.9	10630	21.8
99/00	24245	20.7	9322	24.1
00/01	25273	21.8	9813	25.7

* Human Fertilization and Embryology Authority (HFEA).
References # 8 – 10

Table 2. Results of IVF and ICSI in Europe from cycles initiated in 1997, 1998 & 1999*

	1997		1998		1999	
	IVF	ICSI	IVF	ICSI	IVF	ICSI
Number of transfer cycles	80209	62253	84066	80785	98313	78452
Number of pregnancies	20937	16462	22683	21665	27196	21916
Pregnancy rate per transfer (%)	26.1	26.4	27.0	26.8	27.7	27.9

* European registers by ESHRE
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Table 3. IVF/ART success rates in USA. *

Year	Number of Cycles	Live Birth rate Per Retrieval (%)
1985	3921	5.3
1986	4867	5.2
1987	11806	8.3
1988	17411	9.4
1989	18211	11.3
1990	19079	12.1
1991	24671	12.9
1992	29404	14.2
1993	31900	15.8
1994	26961	18.0
1995	45906	22.8
1996	49586	22.7
1997	55002	23.5
1998	61650	28.9
1999	65751	29.2

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Chapter 2

Aims of the study

The aim of this work is to review the different research projects that have been done by our group in more than one aspect of IVF in an attempt to optimize IVF results. The research is focused on the following areas:

a) The diagnosis and management of the presence of fluid in the uterine cavity in association with hydrosalpinges before starting an IVF cycle.

b) Performing a dummy embryo transfer before the actual transfer to choose the most suitable kind of catheter, measure the length and direction of the uterine cavity, and to diagnose any unanticipated difficulty in the cervix.

c) Studying different factors that may affect the embryo transfer technique such as: the kind of catheter, the presence of cervical mucus, and the presence of air bubbles.

d) Experimenting with the technique of intracytoplasmic sperm injection and investigating the following: 1- the effect of different sperm parameters on the outcome of ICSI. 2- the use of micro-surgically retrieved epididymal and testicular sperm. 3- performing ICSI without cytoplasmic aspiration. 4- the use of ICSI in obstructive and non-obstructive azoospermia.

e) Modification of the technique of multifetal reduction in cases of high order multiple pregnancy in an attempt to improve the outcome.

Chapter 3

Fluid accumulation of the uterine cavity before embryo transfer: a possible hindrance for implantation

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Journal of in Vitro Fertilization and Embryo Transfer, Vol. 8, No. 3, 1991

Abstract

Accumulation of fluid in the uterine cavity was recorded in three cases during vaginal ultrasound (US) monitoring for in vitro fertilization (IVF) cycles. The three patients had hydrosalpinges and tuboovarian cystic masses. They all had a common complaint of intermittent vaginal discharge. Attempts at aspirating the fluid to empty the cavity was done in two cases but the fluid recollected. This condition possibly renders the uterine cavity hostile to the transferred embryos and careful consideration should be taken to diagnose it.

INTRODUCTION

Several factors affect the implantation rate after in vitro fertilization and embryo transfer (IVF-ET). The endometrial thickness and intrauterine environment are important factors. Ultrasound (US) reflectivity of the endometrium and its thickness as a possible parameter for an intrauterine environment favorable for implantation has been studied (1). Changes in the texture and thickness of the endometrium have been observed during follicular maturation in spontaneous and stimulated cycles (2). However, the presence of intrauterine fluid accumulating in its cavity before ET was not observed.

We record three cases showing accumulation of fluid in the uterine cavity before ET.

CASE REPORTS

Case 1

A 27-year old woman complaining of primary infertility came to the Egyptian IVF-ET Center for treatment. She gave a history of peritonitis following hysterosalpingography. She underwent a laparotomy 2 years later and it showed extensive pelvic adhesions and bilateral hydrosalpinges. She was counseled for IVF-ET, and on routine pelvic US examination she was found to have multiple pelvic cystic masses. She also gave a history of vaginal discharge of brownish viscid fluid persistent for many years. The amount of discharge was marked in the second half of the cycle. On clinical examination, the discharge was seen pouring from the cervix. Culture and sensitivity of the discharge were done on more than one occasion and revealed no growth. She received a prophylactic antibiotic course as part of our routine policy. Transvaginal aspiration of the pelvic cysts was done and she received gonadotropin releasing hormone analogue (GnRHa) and human menopausal gonadotropin (hMG)

according to our previously described protocol (3). Transvaginal US ovum pickup resulted in retrieval of six oocytes. It was noted during the ovum pickup that the uterine cavity was distended with fluid (7 x 35 mm) (Fig. la). Attempt at transcervical aspiration of the fluid was done using a Craft ET catheter (R 57.536, Rocket of London, Watford, Herts, England) and 3 ml of dark-brown viscid fluid was emptied. However, before the ET, the uterine cavity was redistended with fluid which was reaspirated immediately before ET and four embryos at the four-cell stage were transferred. Serum hCG 2 weeks later was less than 10 mIU/ml.

Case II

A 30-year-old woman complaining of primary infertility for 5 years was counseled for entry into our IVF program. She had bilateral hydrosalpinges as evidenced from previous hysterosalpingography and laparoscopy. She also complained of brownish vaginal discharge for 3 years. General and pelvic examination revealed no abnormality except for the discharge, which was found to be coming from the uterus. Culture revealed no growth. Aspiration of the hydrosalpinges, prophylactic antibiotics, and ovarian stimulation for IVF were similar to those for case I. On the day of ovum pickup, the endometrial cavity was noted to be distended with fluid (17 x 21 mm) as shown in Fig. 1b. Ovum pickup resulted in retrieval of eight oocytes. Embryo transfer was done 2 days later, replacing four embryos. The uterine cavity was still distended by fluid as during ovum pickup. Serum hCG 2 weeks later was less than 10 mIU/ml.

Case III

A 37-year-old woman with primary infertility for 10 years presented with intermittent brownish vaginal discharge of variable amounts for 8 years. Her general and pelvic examination revealed no abnormalities except for a brownish discharge coming from the

cervix. Laparoscopy diagnosed the presence of bilateral hydrosalpinges and extensive pelvic adhesions. Vaginal US showed multiple cysts. Attempts at aspirating these pelvic cystic masses was done through transvaginal US and 125 ml of fluid was aspirated. The patient was followed up for 2 months after the aspiration and she noted that the vaginal discharge stopped. She was counseled for IVF-ET and ovarian stimulation was induced using GnRHa and hMG. During US monitoring of follicular growth, the uterine cavity was noticed to be distended with fluid (20 x 30 mm) as shown in Fig. 1c, starting from day 10 of the cycle, reaching its maximum on the day of ovum pickup. Transvaginal US ovum pickup resulted in the retrieval of 12 oocytes. Aspiration of the uterine fluid was done, with removal of 5 ml of yellowish clear fluid. Embryo transfer was done 2 days later, replacing four embryos. Pregnancy was not achieved in this cycle. At the time of the transfer the cavity was redistended with fluid but to a lesser degree.

Follow up of the three cases for 6 months following their IVF attempts revealed that the intrauterine fluid accumulated in all cycles. However, the volume of the fluid was slightly less than that accumulated during the IVF cycle. Curettage for endometrial and endocervical biopsies revealed no pathological findings.

DISCUSSION

The presence of pelvic inflammatory cystic masses and hydrosalpinges is a common finding in infertile patients selected for IVF (3). It was also noted that hydrosalpinges may enlarge during stimulation (4). Passage of some fluid into the uterine cavity is likely to occur in some cases. Intermittent hydrosalpinx is a pathological condition in which passage of the fluid from the distended tube into the uterine cavity occurs at different intervals, resulting in copious amount of vaginal discharge (5). The amount could be very minimal to be detected clinically but its effect in rendering the uterine environment hostile to the transferred embryos should be seriously considered. Vaginal ultrasound revealed that the uterine cavity was distended with fluid in these three recorded cases. All of them were diagnosed to have hydrosalpinges and tuboovarian masses and they all had a common complaint of persistent vaginal discharge, which was especially marked during the second half of the cycle. The fluid was not directly related to superstimulation because it was present during the subsequent natural cycles. This finding drove our attention to careful US monitoring of IVF patients who are diagnosed to have hydrosalpinges or tuboovarian masses to spot any fluid in the uterine cavity. The treatment of this condition is rather a difficult one. Transvaginal US aspiration of the fluid in hydrosalpinges and tuboovarian masses before the IVF cycle may be of help (3). Unfortunately, in these three cases the fluid recollected and the discharge recurred. Closing the cornual end of both sides could be worthwhile through laparoscopy or laparotomy after a neosalpingostomy is performed. Bilateral salpingectomy could be done if feasible.

Fig1 (a) Uterine cavity distended with fluid in case 1.

Fig 1 (b) Uterine cavity in case 2.

Fig 1 (c) Uterine cavity in case 3.

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Chapter 4

Dummy embryo transfer: a technique that minimizes the problems of embryo transfer and improves the pregnancy rate in human in vitro fertilization

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Abstract

Three hundred thirty-five patients selected for in vitro fertilization (IVF) were randomly divided into two groups. Group A (n = 167) was subjected to dummy embryo transfer (ET) before the start of IVF treatment to choose the most suitable catheter for each patient. Group B (n = 168) started their IVF treatment without dummy ET. Embryo transfer technique was difficult in 50 cases (29.8%) in group B, whereas no difficulty was met in group A. Pregnancy rate and implantation rate (22.8%, 7.2%) in group A were significantly higher than in group B (13.1%, 4.3%). The lower pregnancy rate in group B is due to the very low pregnancy rate (4%) in difficult ET cases. Dummy ET is a simple procedure that determines the most suitable ET catheter for each patient and avoids unexpected difficult and failed ET.
Fertil Steril 54:678, 1990

INTRODUCTION

The different factors influencing the pregnancy rate after in vitro fertilization and embryo transfer (IVF-ET) have been studied by many investigators in an endeavor to improve results.[1,2] One of the most important factors that determine the outcome of IVF is the technique of ET. This last step of the IVF treatment has been examined by few investigators[2-4].

Most gynecologists involved in IVF programs have certainly noticed the very wide difference in the degree of difficulty in passing the ET catheter through the cervix. Soft ET catheters are preferred over more rigid ones because the latter would be more likely to induce cervical and endometrial laceration.[4] However, passing soft catheters through the cervical canal is often difficult and sometimes impossible. Failed ET and replacing the embryos using a different catheter is very frustrating and might have many adverse effects on the embryos.

Since December 1987 we have been doing a "dummy ET" for patients before starting their IVF treatment to evaluate exactly the passage of the ET catheter through the cervical canal, the length and the direction of the uterine cavity, and to determine the most suitable kind of ET catheter to be used for every patient. This study compares the IVF outcome in patients for whom dummy ET was done and those who underwent IVF treatment without performing dummy ET.

MATERIALS AND METHODS

During the period between December 1987 and October 1989, 335 patients selected for IVF were divided into two groups in a controlled, randomized study on an alternate basis. Group A (n = 167) was subjected to dummy ET before the start of IVF treatment. Group B (n = 168) started their IVF treatment without dummy ET. The clinical characteristics of the two groups are summarized in Table 1. Our protocol for human IVF has been described previously[5].

Dummy ET

After counseling the patient for IVF, the gynecologist performed the dummy ET by introducing a Wallace catheter (1816 N, H.G.; Wallace LTD., Cholchester, England). It is a soft silicon catheter with an external diameter of 1.6 mm and an end opening, fitted in a more rigid outer Teflon sleeve. If the Wallace catheter did not pass easily, a Craft Catheter (R 57.536; Rocket of London, Watford Herts, England) was used. It is a Teflon catheter with an end opening fitted in an introducing cannula that is rigid but malleable. In difficult cases, when neither the Wallace catheter nor the Craft catheter passed easily, a metal cannula was used to pass through the cervical canal. We use a 20-cm metal cannula with internal and external diameters of 1.6 and 1.9 mm, respectively. The cannula curves gently to follow the curvature of the cervical canal. In few cases, when the introduction of the metal cannula was difficult due to very stenosed internal os or anatomical distortion of the cervix, dilatation under anesthesia up to Hegar 12 was done. All the catheters used for the dummy ET were used previously for ET and resterilized.

ET Technique

Embryo transfer was usually done 50 to 60 hours after oocyte pickup. Before the procedure, the patients were given 10 mg Diazepam (Valium; F. Hoffmann-La Roche & Co. LTD, Basle, Switzerland) intravenously. All transfers were done in the dorsal position. The ectocervix and the cervical canal up to the internal os were cleaned of cervical mucus, using a fine sterile cotton swab soaked with culture medium (Ham's F-10; Gibco Laboratories, Grand Island, NY). Meanwhile, the best four embryos were selected for the transfer and were put together in one organ tissue culture dish (Falcon 3037, Cockeysville, MD) containing Hams F-10 media supplemented with 15% heat-inactivated serum. The choice of the ET catheters was as follows:

Group A included 167 patients. The transfer was performed using a Wallace catheter, a Craft catheter, or the metal cannula chosen for each patient according to the previous evaluation of the dummy ET.

Group B included 168 patients who did not previously have dummy transfer. The transfer was done using either Wallace or Craft catheter. The opinion of the gynecologist about the transfer was recorded as (1) easy when the Wallace or Craft catheter passed immediately through the cervix without resistance and (2) difficult when the gynecologist had to use manipulations and strong push to pass the Wallace or the Craft catheter or when they fail to be passed completely and the metal cannula had to be used. The use of a tenaculum to hold the cervix or the occurrence of bleeding was not necessarily considered as difficult transfer. The subjective evaluation of the procedure by the patient was not taken into consideration as there is a wide range of pain perception among patients. This group was divided randomly on an alternate basis into two subgroups.

Group B_1 included 85 cases. The transfer was done using the Wallace catheter. In difficult cases, the metal cannula was used to negotiate the cervical canal, and the Wallace catheter

was threaded into it. If the Wallace catheter was kinked or plugged with mucus, the embryos were replaced in a new one.

Group B_2 included 83 cases. The transfer was done using the Craft catheter. In difficult cases, the metal cannula was used, and the Craft catheter was passed through it. In case of kinking or plugging of the Craft by mucus or blood, the embryos were replaced in a new one.

In all kinds of catheters, the loading was in the following sequence: 15 to 20 μL of medium, 10 μL of air, the embryos were loaded in 15 to 20 μL of transfer medium and withdrawn from the tip of the catheter by aspirating 10 μL of air. The tip of the catheter was placed approximately 0.5 cm from the fundus, and the embryos were gently injected into the uterine cavity. The catheter was immediately checked for retained embryos, blood, or mucus. The length of the uterine cavity was previously measured with ultrasound (US) in group B and directly during the dummy ET in group A. After the transfer, the patients remained in bed for about 4 hours, and they usually stayed overnight in the center and were discharged the next morning. The X2 test was used for statistical analysis.

RESULTS

The dummy transfer showed that the Wallace catheter passed easily in 110 cases (65%). In the remaining 57 patients, the Craft catheter passed easily in 46 cases (80.7%). However, in 11 cases, neither the Wallace catheter nor the Craft catheter were easily introduced and the metal cannula had to be used. This last group included two cases in which the metal cannula did not pass due to severe stenosis and anatomical distortion of the cervical canal, and dilatation under anesthesia up to Hegar 12 was done.

When the ET procedures were done for group A, guided by the previous evaluation of the dummy ET, there was no difficulty met. In group B, there were 118 cases of easy ET and 50 cases of difficult ones. The difficult transfers were met in 32 (37.6%) of ET procedures using the Wallace catheter (group B_1) and in 18 cases (21.7%) of ET procedures using the Craft catheter (group B_2) as shown in Table 2.

The total number of ET procedures was 167 in group A that resulted in 38 pregnancies (22.8%), whereas in group B there were 168 procedures that resulted in 22 pregnancies (13.1%). The difference was statistically significant (P = 0.02). There was no significant difference between the pregnancy rates in the subgroups B_1 and B_2 (Table 2). The total number of embryos transferred in group A were 651 embryos, resulting in 47 implantations (7.2%), whereas in group B, 604 embryos were transferred, resulting in 26 implantations (4.3%). The difference is statistically significant (P = 0.027).

The overall pregnancy rate in all cases of easy ET in both groups was 20.4%, which is significantly higher than the pregnancy rate in cases of difficult ET (4%), p = 0.005. Difficult ET resulted in a significantly lower pregnancy rate compared with easy ET performed using the Wallace catheter, the Craft catheter, or the metal cannula (P = 0.025). In all cases of easy ET, 1,061 embryos were transferred, resulting in 71 implantations (6.7%). In difficult ET, 187 embryos were transferred, resulting in 2 implantations (1%). The difference is highly significant (P = 0.003) as shown in Table 3.

Easy ET, using the Wallace catheter, resulted in a slightly higher pregnancy rate when compared with easy ET using other catheters. The difference, however, was not statistically significant.

DISCUSSION

There is a marked discrepancy between oocyte retrieval, fertilization, and cleavage rates that surpass the 90% range and the pregnancy rate in IVF. There are some mechanical factors that might account for lack of implantation.[6] Careful study of the technique of ET may achieve improvement of the results because minor variations of the transfer technique may impact on the chance of implantation.[7] Our study showed that the pregnancy and implantation rates in group A (22.8%, 7.2%) are significantly higher than in group B (13.1%, 4.3%). Both groups were comparable in all aspects except that ET procedures were easy in all cases of group A due to individual selection of the most suitable catheter for each patient, which was done according to the previous evaluation of the dummy ET. In group B, difficult ET procedures occurred in 29.8% of cases. The lower pregnancy rate in group B is due to the very low pregnancy rate (4%) in difficult ET cases. In these cases, the passage of the catheter was difficult and manipulations had to be used that often resulted in kinking of the catheter or failure of the transfer. In case of failure, the embryos had to be replaced using another more rigid catheter. This certainly exposes the embryos to adverse effects and subjects the gynecologist and the embryologist to a lot of stress. The better pregnancy rate in cases of easy ET corresponds with earlier published data.[2,3]

The other possible value of the dummy ET is the exact measurement of the length of the uterine cavity from the fundus to the external os, which we found more accurate than the US measurement that does not follow exactly the curvature of the uterus. Lack of consideration of high fundal placement of embryos may be partially responsible for the higher rate of spontaneous abortion generally observed with IVF-ET.[8] Also, the dummy ET discovers the anatomically distorted cervical canals and abnormally stenosed ones that might benefit from dilatation under anesthesia before the IVF treatment cycle. In some patients, the pelvic

adhesions pull the uterus acutely toward one side, and the dummy ET is useful to recognize the direction of the cervical canal.

Our results showed that difficult ET procedures had significantly lower pregnancy and implantation rates (4%, 1%) compared with easy ET (20.4%, 6.7%). Soft ET catheters are generally preferred over more rigid ones.[4] This work suggests that the best pregnancy rate was achieved when the softest catheter (Wallace) was used and passed easily through the cervical canal. Unfortunately, these soft catheters resulted in the highest rate (37.6%) of difficult ET procedures with its consequences of lowering the pregnancy rate. Therefore, soft catheters are preferred but only when its easy passage through the cervix is assured through the dummy ET.

Most IVF programs aim at the choice of the most suitable catheter to be used for their patients to achieve the best results. The choice should be individualized for each patient by performing the dummy ET, which is a very simple procedure that avoids the occurrence of unexpected difficult and failed embryo transfers.

Table 1 Clinical Characteristics of the Patients

	Group A	Group B	
		B_1	B_2
No. of patients	167	85	83
Age (mean ± SD)[a]	33.98 ± 4.05	34.47 ± 4.24	32.91 ± 5.42
Indication for IVF:			
Tubal	145	74	73
Tubal + male	11	6	5
Endometriosis	4	2	3
Tubal + ovulatory	7	3	2
Duration of infertility in years (mean ± SD)	9.92 ± 4.67	8.50 ± 3.91	8.86 ± 4.72

[a] SD, standard deviation.

Table 2 Results of ET in Group A (Previous Dummy ET) and Group B (No Previous Dummy ET)

	Total no. of ET	No. of embryos per transfer[a]	No. of pregnancies	Pregnancy rate	No. of difficult ET
				%	
Group A	167	3.9 ± 1.2	38	22.8[b]	—
Group B	168	3.6 ± 1.9	22	13.1[b]	50 (29.8)[c]
B_1	85	3.7 ± 1.5	10	18.8	32 (37.6)
B_2	83	3.5 ± 2.9	12	14.5	18 (21.7)

[a] Values are means ± SD.
[b] $P = 0.02$; $\chi^2 = 5.32$ with 1 degree of freedom.
[c] Values in parentheses are percents.

Table 3 Implantation Rates in Different Groups

	No. of ET	Total no. of embryos transferred	No. of implantations	Implantation rate
				%
Group A	167	651	47	7.2[a]
Group B	168	604	26	4.3[a]
Easy transfers in both groups	285	1061	71	6.7[b]
Difficult transfers in both groups	50	187	2	1[b]

[a] $P = 0.027$.
[b] $P = 0.003$.

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Chapter 5

Dummy embryo transfer using methylene blue dye

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Human Reproduction vol. 9 no.7 pp.1257-1259, 1994

Abstract

The aim of this prospective work was to evaluate different catheters and techniques used for embryo transfer. Studies were performed on 105 IVF patients before the start of treatment cycles. Each patient was used as her own control to study the expulsion of methylene blue (MB) dye after dummy embryo transfer. Group A (n = 35) underwent the test twice, before and after aspiration of the cervical mucus. Group B (n = 30) underwent the test twice with and without the presence of two air bubbles in the embryo transfer catheter. Group C (n = 40) underwent the test twice using two different catheters, the Wallace and the Craft catheters. The results showed that the dye was extruded at the external os in 57% of the cases when the cervical mucus was not aspirated compared to 23% when the mucus was aspirated ($P = 0.01$). The dye was extruded in 33% of the cases with air bubbles in the catheter as compared to 27% when no air was present ($P > 0.05$). When the Wallace catheter was used expulsion occurred in 25.5% compared to 77.5% when the Craft catheter was used. We concluded that using soft catheters and complete aspiration of cervical mucus significantly reduced the expulsion of the dye. The presence of air bubbles did not affect rate of expulsion of the dye.

INTRODUCTION

The technique of embryo transfer is one of the most critical steps affecting the success rate after in-vitro fertilization (IVF). We previously reported that performing a dummy embryo transfer for patients before starting their IVF treatment significantly improved the pregnancy rate (Mansour et at., 1990). The possibility of embryo expulsion after what was thought to be a successful transfer has been the concern of different investigators (Kerin et at., 1981; Poindexter et at., 1986; Schulman, 1984). Were the embryos transferred to the uterus? And if they were, did they stay there or were they expelled? This study was designed to answer some of these questions. The aim of the present work was to evaluate different catheters and different techniques for dummy embryo transfer in which methylene blue (MB) dye was used in place of the embryo column.

MATERIALS AND METHODS

This prospective study was done on 105 IVF patients before the start of their treatment cycles. It was done 4-5 days after the detection of the luteinizing hormone (LH) surge in urine, which is approximately the time of embryo transfer in the subsequent IVF cycle. The patients signed a consent to undergo this test as a rehearsal of their actual embryo transfer with the aim of improving the IVF results. Each patient was used as her own control to study the rate of expulsion of MB after dummy embryo transfer in relation to three factors.

Group A: the effect of cervical mucus

A dummy MB test was done twice on 35 patients using the Wallace embryo transfer catheter (catalogue number 1816 N; H.G. Wallace Ltd, Colchester, UK). It is a soft silicon catheter with an external diameter of 1.6 mm and an end opening, fitted in a more rigid outer Teflon sleeve. The catheter was loaded with MB as shown in Figure la, and the transfer itself was done by the same gynaecologist using two different techniques: (i) wiping the cervical mucus from the surface of the cervix only and (ii) complete aspiration of the mucus from the cervical canal using a tuberculin syringe and a previously used re-sterilized Wallace catheter. In both methods the catheter was gently introduced without grasping the cervix with a tenaculum, and a volume of 20-25 µl MB dye was injected.

Group B: the effect of air bubbles

The Wallace catheter was used twice on 30 patients loading the catheter with two different methods: (i) loading no air in the catheter as shown in Figure la, and (ii) using air bubbles in the catheter before and after the MB as shown in Figure lb. The total volume was the same in both conditions and the transfer was done as described above.

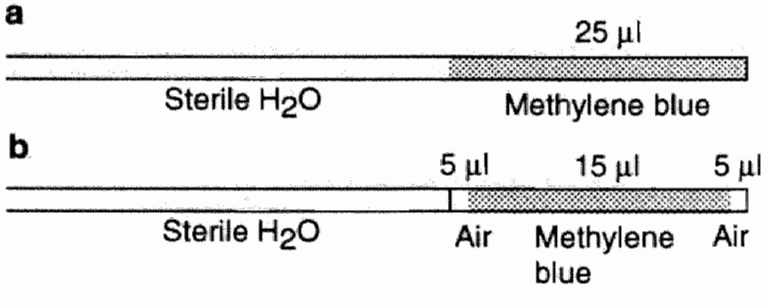

Fig. 1. Loading of the catheter for dummy embryo transfer. (**a**) Methylene blue only; (**b**) methylene blue and air bubbles.

Group C: the effect of different catheters

A dummy MB test was done twice for 40 patients using two different catheters: (i) the Wallace catheter and (ii) the Craft catheter (R 57.536; Rocket of London, Watford, Herts, UK). It is a Teflon catheter, that is more rigid than the Wallace catheter, with an end opening and fitted in an introducing catheter that is more rigid but malleable.

In both kinds of catheters, the loading was done as shown in Figure la, and the transfer was done by the same gynaecologist as described previously. In all groups the catheter was gradually withdrawn and the cervix was observed for a few minutes to visualize the dye at the external os; a positive result was recorded if the dye was extruded or a negative one if the dye was not extruded. Cases with difficult transfers or the ones with bloody spots at the external os were excluded from the study.

Statistical analysis

McNemar's test was used.

RESULTS

In group A, the dye was visualized at the external os in 20 cases (57%) when the cervical mucus was not aspirated compared to eight cases (23%) when the mucus was aspirated ($P = 0.01$).

In group B, the dye was extruded in eight cases (27%) when no air was loaded in the catheter compared to 10 cases (33%) when air was present ($P > 0.05$).

In group C, the dye was extruded at the external os in nine cases (22.5%) when the Wallace catheter was used compared to 31 cases (77.5 %) when the Craft catheter was used ($P < 0.001$). Extrusion of the dye occurred in 42% of all cases of dummy embryo transfer in the three groups using different techniques.

DISCUSSION

Embryo transfer technique is one of the most important factors affecting IVF results. The assurance that the embryos are successfully transferred to the uterus and that they remain in the uterine cavity is an essential prerequisite for successful IVF results.

The subject of embryo transfer has been described in detail by Betteridge and Rieger (1993), including the synchronization of reproductive cycles and techniques. Embryo transfer is being done routinely through the cervix and there are only a few reports on the use of the transfundal surgical route (Parsons et al., 1987; Kato et al., 1993). Therefore, it is essentially a blind technique following which there is no certainty that the embryos are even within the uterine cavity. The mere introduction of the embryo transfer catheter through the cervical canal may initiate uterine contraction. A forceful expulsion of a droplet of fluid was noticed at the external os up to 5 - 10 min after embryo transfer (Schulman, 1984). Consequently the transferred embryos are probably being partially or totally lost. Poindexter et al. (1986) collected 15% of the transferred embryos after expulsion from the external os, on the vaginal speculum, and the outer surface of the catheter. In fact, the lost fraction is probably > 15% as it is difficult to find the extruded embryos. It has been shown that stimulation of the cervix causes the release of oxytocin, thus increasing uterine contractions, and that an injection of oxytocin induces uterine contractions at all stages of the oestrous cycle in the cow (Harper et

al., 1961). In an early study on cow, a technique dependent on the use of 'artificial ova' consisting of resin spheres impregnated with radioactive gold was used. At varying times after the insertion of the spheres, the uterus, cervix and vagina were checked for radioactivity. It was found that after 1.5 h, a large proportion of the spheres had been expelled from the uterus altogether (Harper et al., 1961). In a similar study in humans by Knutzen et al. (1992), using radio-opaque dye mimicking embryo transfer, it was found that the dye remained primarily in the uterine cavity in only 58% of cases, and it was concluded that the remainder of the patients would have lost their opportunity for pregnancy as a result of the embryo transfer procedure. The role of embryo transfer and its associated difficulties on the outcome of human IVF were examined to assess any effects of the smooth muscle relaxant, glyceryl trinitrate (Shaker et al., 1993). In an attempt to avoid embryo expulsion and ectopic pregnancy, Feichtinger et al. (1992) used a two-component fibrin sealant in human embryo transfer. Their technique resulted in a significant reduction in ectopic pregnancy rate. Our study showed that the dye was visualized at the external os in 42% of all cases. This means that the uterus extruded the dye, at least partially, which was transferred using the same volume and technique as in actual embryo transfer. Consequently, it is possible that the embryos may be extruded partially or totally at the time of embryo transfer. The rate of extrusion was significantly less when using soft catheters compared to rigid ones. Therefore, soft catheters are generally preferred over more rigid ones, as shown previously by Wisanto et al. (1989). Still with the use of the soft catheters, there was extrusion of the dye in 25.5% of cases. There was no significant difference between the presence or absence of two air bubbles as long as the volume was not increased. Removing the cervical mucus before embryo transfer significantly reduced the appearance of the dye at the external os. Due to its high elastic nature, the cervical mucus probably stretches with the embryo transfer catheter during the introduction and goes back outside during withdrawal of the catheter trapping the

MB with it. Therefore, we think it is of importance to clear the cervical mucus completely before embryo transfer, taking into consideration that it may take up to 10 min.

We conclude that using soft catheters for embryo transfer and complete aspiration of cervical mucus significantly reduced the expulsion rate of the dye. The presence of air bubbles did not have a significant effect provided that it did not increase the volume injected. Observations from this study and from previous work, showing that almost 40% of all patients might have lost their chance of pregnancy at the time of embryo transfer, should alarm physicians and scientists working in the field, and stimulate extensive research to ensure safe transfer of the embryos to the uterine cavity and to prevent factors which might cause their expulsion.

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Chapter 6

The effect of sperm parameters on the outcome of intracytoplasmic sperm injection

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Fertility and Sterility Vol. 64, No.5, November, 1995

Abstract

Objective: To investigate the influence of sperm parameters on the fertilization and pregnancy rates in intracytoplasmic sperm injection (ICSI).

Design: A retrospective analysis of 130 cycles of ICSI performed for the treatment of male factor infertility.

Setting: The Egyptian IVF-ET Center.

Participants: One hundred thirty couples with the diagnosis of male factor infertility or with previous failed fertilization in conventional IVF or sub zonal sperm injection.

Intervention: Ovum pick-up and ICSI.

Main Outcome Measure: Fertilization and pregnancy rates in relation to different semen parameters.

Results: A total of 1,433 oocytes were retrieved and 1,071 metaphase II oocytes were injected. Normal fertilization occurred in 620 oocytes (58%). Embryo transfer was done for 128 (98.5%) patients, and a total of 46 (35%) clinical pregnancies were achieved. There was no statistically significant difference in the fertilization or pregnancy rates between patients who had previously failed fertilization in conventional IVF, patients with subfertile semen, patients with semen between 1 and 10 X 10^6/mL, and patients with semen <1 X 10^6/mL. There was also no significant difference in the fertilization and pregnancy rates between patients with <95% or >95% teratozoospermia.

Conclusion: In ICSI, the fertilization and pregnancy rates are not affected by different semen parameters as long as morphologically well-shaped live sperms could be used for the injection. Fertil Steril 1995;64:982-6

INTRODUCTION

Different modalities of assisted reproduction for the treatment of male factor infertility including conventional IVF were tried for several years with little success (1). Various techniques of micromanipulation have been developed to assist fertilization, including partial zona dissection and subzonal insemination (SUZI) (2,3). Palermo et al. (4) reported the first successful pregnancy after intracytoplasmic sperm injection (ICSI) for the treatment of male factor, and in a large series Van Steirteghem et al. (5) reported a higher success rate using ICSI as compared with SUZI.

After this report, most IVF centers, including ours, shifted to the use of the ICSI technique as the only method of assisted fertilization. The introduction of ICSI opened the door for the treatment of male factor infertility irrespective of the severity of the condition. The aim of the present work was to study the effect of different sperm parameters on the fertilization and pregnancy rates after ICSI.

MATERIALS AND METHODS

A series of 130 ICSI cycles was performed for the treatment of infertility due to male factor with different degrees of severity. The mean age of the female partners was 35 ± 4 (mean ± SD) years (range 23 to 41). The mean duration of infertility was 8.3 ± 3.9 years (range 3 to 19). Couples were counseled extensively about the technique and signed a consent form prepared by our ethical committee. The initial semen evaluation was done on at least two samples before the treatment cycle and one or both samples were cryopreserved. Semen was

considered abnormal if the count was <20 X 10^6/mL, motility <40% according to the World Health Organization (WHO) criteria (6), and normal morphology <14% according to Kruger et al.'s (7) strict criteria.

The patients were divided into four groups according to semen parameters. Group 1 (n = 28) included patients who had previous total failure of fertilization, at least twice, in regular IVF with normal semen parameters, including 5 patients with previous failure or poor fertilization after SUZI. Group 2 (n = 21) included patients who had subfertile semen that was just below the WHO criteria. They had a count of 10 to 20 X 10^6/mL, motility <40%, and abnormal forms >40%. Group 3 (n = 32) consisted of patients who had a count from 1 to 10 X 10^6/mL, motility <30%, and abnormal forms >60%. Group 4 (n = 49) included patients who had a count <1 X 10^6/mL, including 28 patients with only a few motile sperms in the whole ejaculate. In this latter subgroup the actual counts were not evaluated so as not to lose any motile sperms in the counting chambers. These patients were routinely asked to provide a second and, if possible, a third sample. We used our routine long GnRH analogue and hMG protocol for the induction of ovulation as described before (8).

Oocyte Handling

The oocytes were denuded of their surrounding cumulus cells (2 hours after ovum pick-up) using hyaluronidase 80 IU/mL in N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-buffered Earle's Balance Salt Solution (EBSS; cat. no.1200; Medicult, Copenhagen, Denmark) for 10 to 15 seconds, and then the oocytes were transferred to Ham's F-10 (GIBCO Laboratory, Grand Island, NY) for complete removal of the corona cells by repeated aspiration in a finely pulled pipette. The oocytes were then rinsed and incubated in Ham's F-10 medium/10% patient serum under mineral oil (Squibb, Princeton, NJ) until the

time of injection (2 to 3 hours after pick-up) that was done only for oocytes that extruded their first polar bodies, metaphase II (MII).

Semen Processing

Semen samples were usually collected before or at the time of ovum pick-up, and when necessary the husband was asked to collect a second sample a few hours later. Five milligrams of alpha chemotrypsin (cat. no. C-4129; Sigma Chemical Company, St. Louis, MO) in 2 mL Ham's F-10 was added to the semen sample. After complete liquefaction, the semen was washed twice with Ham's F-10, and the final pellet was resuspended in 0.2 mL medium and layered carefully in the surrounding ring of a Tea tube (9), filled with Ham's F-10, and incubated until the time of injection. Sperm suspension was aspirated from the central cone of the Tea tube, and the count was adjusted from 2 to 6 X 10^6/mL. Approximately 2 μL from this sperm suspension was added to the polyvinylpyrrolidone (PVP) droplet. In cases of severe oligoasthenospermia, the micropipettor was used to aspirate 2 μL from the bottom of the central cone of the Tea tube to be added directly to the PVP droplet. We always have been able to retrieve enough sperms for the microinjection using this technique of sperm processing, and we have never used Percoll gradient. In some cases of very severe oligospermic samples, a microdroplet from the pellet itself had to be used to search for a motile sperm and transfer it to the PVP droplet using the microinjection pipette.

Microinjection Procedure

The cover of an organ tissue culture dish (Falcon 3037; Becton Dickinson Labware, Lincoln Park, NJ) was used as the injection dish. A microdroplet of 10 μL PVP 10% (cat. no.1089;

Medicult, Copenhagen, Denmark) was put in the middle and was surrounded by eight microdroplets of EBSS/HEPES (cat. no.1034; Medicult) under oil.

The oocytes were put individually in the EBSS/ HEPES droplets, and the sperms were put in the PVP droplet as described above. The microinjection procedure was done using an inverted phase microscope (diaphot TMD; Nikon, Tokyo, Japan) with a heated stage (Swemed Laboratory, Frolunda, Sweden) and equipped with a Hoffman condenser (Modulation Optics Inc., Greenvale, NY), and micromanipulation was set (Narishige Inc., Tokyo, Japan). The procedure was allowed to be followed on a monitor (model KX-14CPI; Sony, Tokyo, Japan) connected to a video camera (Panasonic color CC TV camera model WVCL 300/6; Matsushita Communication Industrial Co., Tokyo, Japan) that was attached to the microscope. The injection micropipettes were ready made (Humagen Fertility Diagnostics, Charlottesville, VA); they had an inner diameter of approximately equal to 7 μm, a bevel of 45°, and a spike. The holding pipettes were from Cook (cat. no. K- HPIP-1000; Eight Mile Plains, Queensland, Australia).

The microinjection pipette was lowered in the PVP microdroplet, and one sperm was chosen (the best available motile and morphologically well shaped) and was immobilized by touching its tail near the midpiece with the injection micropipet. The immobilized sperm was aspirated, tail first, into the injection pipette. After securing the oocyte in position with the holding pipette (polar body at 6 or 12 o'clock position), the injection pipette was introduced at the 3 o'clock position through the zona pellucida, oolemma, and deeply into the cytoplasm, and the sperm was injected slowly and the pipette was withdrawn. We never tried to aspirate the cytoplasm. After injection, the oocytes were rinsed and incubated under oil in Ham's F-10/10% serum.

The next morning, the oocytes were examined for evidence of fertilization and transferred to fresh media (Ham's F-10/20% patient serum). Embryo transfer was done on day 2 after the

pick-up, using a Wallace catheter (cat. no.1816; N.H.G. Wallace Limited, Cholchester, England) or a Labotect catheter (Labotect, Bovender-Gottingen, Germany) if the Wallace catheter could not be introduced. Luteal phase support was given routinely in the form of 2,500 IU hCG every 4th day. Cases that were considered at high risk of developing ovarian hyperstimulation syndrome were given a daily P injection (100 mg, P USP; STERIS, Phoenix, AZ). Serum (β-hCG test was done 2 weeks after the ET to diagnose pregnancy, and ultrasonic examination was done after 2 to 3 weeks for patients with a positive test. Clinical pregnancy was diagnosed by the presence of a gestational sac with fetal echoes and pulsations. Chi-square test was used for statistical analysis. $P < 0.05$ was considered significant.

RESULTS

A total of 130 cycles were performed, and 1,433 oocytes were retrieved. Out of 1,433 oocytes retrieved, 1,071 (74.7%) were at the metaphase II, 172 (12%) were at the metaphase I, 143 (10%) were at the prophase, and 47 (3.3%) were fractured and ill formed. Out of 1,071 oocytes at the metaphase II that were injected, 1,028 (96%) were intact after the injection and when examined the next morning. Normal fertilization (two pronuclei) occurred in 620 oocytes (58%), 3 PN were observed in 10 (1%), and 1 PN was observed in 32 (3%). Twenty-six oocytes were already at the two-cell stage 18 to 20 hours after the injection. Cryopreservation was done for 133 normally fertilized oocytes (21.5%), and cleavage arrest occurred for 39 (8%) of the normally fertilized oocytes. Out of the 130 ovum pick-up cycles, 128 (98.5%) reached the ET stage with an average of 3.5 embryos per transfer. A total of 46

(35%) clinical pregnancies were achieved, including 12 (26%) multiple pregnancies. A total of 10 abortions (21%) occurred in this series.

The number of oocytes retrieved, number of metaphase II oocytes that were injected, number of oocytes fertilized, and the clinical pregnancies in all groups are shown in Table 1. The fertilization rates were 52%, 58%, 59%, and 61% in groups 1, 2, 3, and 4, respectively. The clinical pregnancy rates were 36%, 38%, 34%, and 35% in groups 1, 2, 3, and 4, respectively. There was no statistically significant difference in fertilization and clinical pregnancy rates between the four groups of patients irrespective of the semen parameters. There was no significant difference in the pregnancy wastage between all groups. As shown in Table 2, there was no statistically significant difference in the fertilization and pregnancy rates between patients with $\geq$ 95% abnormal forms (n = 41) and patients with <95% abnormal forms (n = 89).

In 122 of 130 patients, enough numbers of motile morphologically well-shaped sperms have been identified and used for microinjection. Eight patients had 100% teratozoospermia, and abnormal forms were the only available sperms for the injection. Two patients had 100% amorphous heads, and there was a complete failure of fertilization. In 6 patients, with 100% midpiece abnormalities, 54 metaphase II oocytes were injected and normal fertilization was achieved in 31 (57%). Fertilization was achieved in all 6 patients, and 4 of them became pregnant. Two patients aborted and the two other pregnancies are ongoing.

DISCUSSION

In conventional IVF it was reported that embryos resulting from poor semen had less quality and were associated with low pregnancy rate (10). Before the introduction of ICSI, it had

been shown that sperm characteristics influenced the outcome of assisted fertilization techniques and that sperm of mostly teratozoospermic semen appeared to be unable to produce functionally normal embryos either because of metabolic errors or genetic aberrations (11). Palermo et al. (12) investigated the relation between the sperm parameters and the outcome of SUZI and ICSI and found out that none of the single sperm parameters, such as concentration, progressive motility, or morphology, correlated with the outcome of assisted fertilization. On the other hand, they found a correlation between the number of progressive motile sperms with normal morphology in the whole ejaculate and the fertilization and pregnancy rates. They also demonstrated that embryos obtained from men with extensive teratozoospermia implanted at a significantly lower rate than embryos from men with less severe teratozoospermia; however, they commented that these findings required confirmation on a larger number of pregnancies.

The present study using ICSI for the treatment of male factor infertility showed that there was no correlation between the sperm motility, density, or percentage of abnormal forms and the fertilization and pregnancy rates so long as motile morphologically well-shaped sperm could be found for the microinjection. Our data support other recent publications that showed no relation between semen parameters and the ICSI results. Cohen et al. (13) reported that in ICSI there was no significant correlation between the percentage of normal sperm forms, fertilization, and implantation. They were also not able to correlate fertilization and implantation with sperm concentration or motility. The same finding was also demonstrated by Payne et al. (14), who reported that there was no significant difference in the fertilization or pregnancy rates between patients who had only occasional motile spermatozoa in the ejaculate, semen that was too poor for routine IVF, or had failed routine IVF and/or SUZI.

In agreement with several recent reports (12-14), our data demonstrated that patients who had previous failed fertilization with conventional IVF or SUZI had a similar success rate as compared with other groups. Rowlands et al. (15) reported that true idiopathic fertilization failure must be consistent and not a haphazard event that may rectify itself in subsequent cycles. It is our routine policy that patients with normal semen and only one cycle of previous failure of fertilization in conventional IVF not to be allocated directly for our ICSI program. In their next IVF cycle, the retrieved oocytes for each patient are randomly divided between conventional IVF and ICSI. This will confirm the diagnosis and at the same time will rescue the cycle with embryos resulting from the ICSI group. In this study, the first group consisted of patients that had at least two cycles of previous failure of fertilization.

Our results show that there was no significant difference in fertilization and pregnancy rates in patients with severe teratozoospermia as compared with less-severe forms (Table 2). This could be explained by the fact that we were able to inject morphologically well-shaped live sperm in all but eight patients with 100% teratozoospermia. It is believed that the outcome of ICSI is not affected by the different sperm parameters if a well-shaped motile sperm could be isolated and injected, and the number finally needed for injection in ICSI is very small.

In patients with 100% teratozoospermia, there was complete failure of fertilization in the two patients with amorphous sperm head. On the other hand, there was an excellent fertilization and pregnancy rate in patients with midpiece deformities. Midpiece deformity is probably due to the presence of cytoplasmic droplet that seems to have no deleterious effect on the fertilizing ability of the spermatozoa as long as the head is normal. At the time being, in patients with 100% teratozoospermia, it is recommended to choose sperm for injection with midpiece abnormalities rather than head defects if at all possible. However, the data on absolute teratozoospermia are too small to confirm this finding. Lunden et al. (16) reported recently the first delivery from patients with 100% acrosomeless sperm (rounded heads).

This shows that even in patients with 100% abnormal forms, pregnancy and delivery of healthy babies is possible after the use of ICSI.

A Percoll gradient was not used in this study to select spermatozoa used for injection. The sperm selection method used in this study, which is routinely used in our ICSI program, is unusual when compared with the majority of other ICSI programs. It may have an interesting implication in the comparison of embryo cleavage, quality, implantation, and normality of the babies born. This experience may clarify the different outcomes in studies that use a sperm selection by Percoll where silica gel particles may have been injected into the egg during ICSI.

In conclusion, our results show that the excellent success rate of ICSI, which was reported, recently is reproducible. It also shows that different sperm parameters do not affect the fertilization, pregnancy rates, or the outcome of pregnancy as long as a morphologically well-shaped motile sperm is used for the injection. Further studies on patients with total teratozoospermia are ongoing to evaluate the fertilization and pregnancy rates and the outcome in this group.

Table 1 Fertilization and Pregnancy Rates in All Groups

Group	No. patients	Oocytes retrieved	Oocytes injected (metaphase II)	Oocytes fertilized* (2PN)	Clinical pregnancies* (rate)
1	28	302	252	132 (52)†	10 (36)‡
2	21	233	167	96 (58)	8 (38)
3	32	347	256	151 (59)	11 (34)
4	49	551	396	241 (61)	17 (35)
Total	130	1,433	1,071	620 (58)	46 (35)

* Values in parentheses are percents.
† No significant difference between any of the groups, $P = 0.260$.
‡ No significant difference between any of the groups, $P = 0.999$.

Table 2 Fertilization and Pregnancy Rates in Relation to Abnormal Form Rate

Percentage of abnormal forms	No. patients	No. oocytes injected (metaphase II)	No. fertilized oocytes* (2PN)	No. pregnancies* (rate)
>95%	41	349	206 (59)†	15 (36.6)‡
<95%	89	722	414 (57.3)	31 (34.8)

* Values in parentheses are percents.
† No significant difference between the two groups, $P = 0.647$.
‡ No significant difference between the two groups, $P = 0.998$.

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Chapter 7

Intracytoplasmic sperm injection using microsurgically retrieved epididymal and testicular sperm

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FERTILITY AND STERILITY Vol. 65, No.3, March 1996

Abstract

Objective: To evaluate treatment of couples who are infertile due to unreconstructable obstructive azoospermia with microsurgical epididymal sperm aspiration or testicular sperm and intracytoplasmic sperm injection (ICSI).

Design: Prospective.

Setting: The Egyptian IVF-ET Center in association with Cairo University.

Patients: Twenty-three couples who are infertile due to obstructive azoospermia in which reconstructive microsurgery failed or was not possible.

Interventions: Microsurgical epididymal or testicular sperm retrieval and ICSI in 24 cycles.

Main Outcome Measures: Fertilization and pregnancies.

Results: All 24 cycles had successful fertilization and reached the ET stage. In microsurgical epididymal sperm aspiration cases, fertilization rate per metaphase II oocyte was 63% (109/172). Six patients became pregnant, including one set of twins and one set of triplets. One pregnancy resulted from the use of cryothawed epididymal sperm. In testicular sperm aspiration cases, the fertilization rate was 59% (32/54) and four clinical pregnancies resulted.

Conclusion: The use of microepididymal sperm aspiration or testicular sperm aspiration in conjunction with ICSI provide a highly precise and efficient procedure for achieving pregnancy in cases of unreconstructable obstructive azoospermia. Fertil Steril 1996; 65:566-72

INTRODUCTION

Patients with obstructive azoospermia due to congenital absence of the vas deferens or those who failed reconstructive surgery were considered hopelessly infertile. Recently, IVF in conjunction with microsurgical sperm aspiration, first described in 1984 by Pryor et al. (1) and in 1985 by Temple-Smith et al. (2), provided new hope for those patients. Pregnancy and live birth after microepididymal sperm aspiration and IVF was reported by Silber et al. (3, 4) in 1988 and 1990. The fertilization and pregnancy rates after microepididymal sperm aspiration and IVF were low and epididymal sperm appeared to have impaired fertilizing ability with conventional IVF (5). The introduction of intracytoplasmic sperm injection (ICSI) (6) made IVF so efficient and precise to the extent that very low numbers of spermatozoa, regardless of their motility pattern, are required to fertilize the oocytes retrieved for the procedure. The combination of both microepididymal sperm aspiration and ICSI proved to be a highly successful technique that significantly improved the results as reported by Tournaye et al (7). Silber et al. (8) demonstrated clearly that ICSI could achieve better results than conventional IVF with microsurgically retrieved epididymal or testicular sperm. In some cases, the epididymis is absent or fibrosed, and a testicular biopsy is needed to extract spermatozoa. Recently, fertilization and pregnancies were reported after the use of testicular sperm in conjunction with ICSI (9). The aim of the present work was to report our experience with the first 24 cycles of ICSI using microsurgically retrieved epididymal or testicular sperm.

MATERIALS AND METHODS

A total of 23 couples with infertility due to obstructive azoospermia were considered for microsurgical sperm retrieval in conjunction with ICSI. Eight men had bilateral congenital absence of the vas deferens. Alloplastic spermatocele had been implanted in one of them but aspiration from this artificial spermatocele was negative for sperm. Two cases had single testis with unilateral congenital absence of the vas in one patient and acquired vasal obstruction in the second patient. Three men had unilateral absent vas with contralateral ductal obstruction for which previous attempts of reconstructive surgery had failed. Eight patients had acquired obstructive azoospermia and previous epididymovasostomy trials had failed due to multiple obstructions. One patient had a previous vasectomy operation for contraception and failed reconstructive surgery twice. One patient had unilateral orchidopexy, the other testis was normal. His semen showed severe oligospermia (<1 X 10^6/mL). Bilateral testicular biopsy showed germinal aplasia at the site of orchidopexy and normal spermatogenesis on the other testis. Incomplete obstruction was diagnosed and the patient was appointed for ICSI. On ovum pick-up day, semen analysis showed azoospermia. Testicular sperm retrieval was done as an emergency to avoid canceling the ICSI procedure. The female partners had regular ovulatory cycles and normal pelvic and general examinations. There was no history suggestive of previous pelvic infection or pelvic surgery. An infertility work-up and tubal patency test were not done routinely for them. Patient characteristics are shown in Tables 1 and 2.

Counseling was done for each of the couples with congenital absence of the vas deferens to inform them about the possibility of having offspring with cystic fibrosis (CF). They were told that if the wives were CF negative, then the risk of having offspring with CF was approximately 0.3% (10). None of the patients agreed to be tested because they considered the risk to be minimal.

Ovarian Stimulation and Oocyte Retrieval

The wives received 200 µg/8 h Suprefact nasal spray (Hoechst AG, Frankfurt am Main, Germany) 10 days before menstruation and until the day of hCG injection. Human menopausal gonadotropin (Humegon; Organon, Oss, The Netherlands) was given (2 to 3 weeks after the start of Suprefact) 150 IU/d IM for 5 days, and then the dose was modified according to the response. When two or more follicles reached >18 mm in mean diameter, 10,000 IU hCG was given IM and ovum pick-up was scheduled 36 hours later.

Microsurgical Epididymal Sperm Aspiration

The procedure was performed under general anesthesia in an ambulatory surgicenter as an outpatient procedure. Via a small scrotal longitudinal incision, the tunica vaginalis was identified and incised; subsequently, the epididymis was delivered into the operative field. At this point, the procedure was performed under the operating microscope at lOx to 40x magnification as a modification of the technique described by Silber et al. (3). The aspiration was attempted at the lowest possible level within the epididymis. The epididymal capsule was incised using microscissors and epididymal fascia was dissected. Meticulous hemostasis was achieved using a bipolar microcoagulator on low-voltage setting. A single epididymal tubule was isolated and punctured using the microscissors. The fluid coming out from the open tubule was aspirated with a tuberculin syringe containing 0.1 mL of HEPES Earl's buffered salt solution (EBSS, cat. no.1034; Medicult, Copenhagen, Denmark) attached to a 25-gauge angiocatheter. The fluid was examined microscopically within the operating room for the presence of motile sperms. If the aspirated fluid contained no motile sperm, another tubule in a more proximal site was incised. When the aspirate contained motile sperms it was transferred to a 5-mL Falcon tube containing 1 mL HEPES-buffered EBSS media. The same

syringe was used for continued aspiration at the same site until a sufficient number of sperm was obtained. The epididymal sheath was closed with interrupted 8-0 nylon sutures. This closure of the tunica epididymis would decrease the amount of postoperative scarring and adhesions, thus allowing repeat microepididymal sperm aspiration to take place if necessary. In seven cases, testicular biopsies were taken because the epididymis was fibrosed or absent. The closure of the tunica vaginalis, dartos, and skin was carried out in a standard fashion.

The Falcon tubes (Becton Dickson, Plymouth, United Kingdom) containing the aspirated epididymal sperms or the testicular biopsies were put in an isolated container to prevent exposure to sunlight or extremes of temperature. The container was carried to our IVF center and the delivery time ranged from approximately 0.5 to 1 hour.

Epididymal Sperm Processing

The epididymal sample was washed with HEPES EBSS and centrifuged for 7 minutes at 400 X g. The pellet was resuspended in 0.2 mL media and layered carefully in the surrounding ring of a Tea tube (11) that was filled with Ham's F-10 medium (GIBCO Laboratory, Grand Island, NY) and 10% patient serum. The tube was incubated at 5% CO_2 at 37°C until use. After completing the microinjection, the rest of the sperm was cryopreserved in all cases for possible future use.

Testicular Biopsy Processing

The biopsy was morselized in a petri dish (Falcon cat. no.3001, Becton Dickson) under the dissecting microscope using two needles. The morselized tissues were incubated in 1.5 mL Ham's F-10 medium for approximately 2 hours in a 5-mL Falcon tube. The contents of the

tube then were mixed and allowed to settle for 1 minute and the deposited pieces of testicular tissue were removed.

The tube was centrifuged for 5 minutes at 400 X g and the pellet was resuspended in 0.2 mL Ham's F-10 medium. Two or three microdroplets were used from this resuspended pellet to be put near the PVP microdroplet in the injection dish. Using the injection micropipette, a search was done for a motile sperm, which was aspirated from among Sertoli cells, red blood cells, and debris and transferred to the polyvinylpyrrolidone (PVP) droplet. It was immobilized, aspirated tail first, and injected into the oocyte. During sperm immobilization by touching the sperm near the midpiece, cytoplasmic droplets and some debris around the midpiece and tail were removed.

Oocyte Handling

The oocytes were denuded of their surrounding cumulus cells 2 hours after ovum pick-up using 80 IU/mL hyaluronidase (catalog no.1200; Medicult) for 10 seconds, and then the oocytes were transferred to Ham's F-l0 for complete removal of the corona cells by repeated aspiration in a finely pulled pipette. The oocytes then were rinsed and incubated in Ham's F-10 and 10% patient serum under mineral oil (Squibb, Princeton, NJ) until the time of injection. Intracytoplasmic injection was done only for oocytes that extruded their first polar bodies.

Microinjection Procedure

The injection dish used was the cover of a Falcon dish (cat. no.3037; Becton Dickinson, Plymouth, United Kingdom). A microdroplet of 10 µL PVP 10% (cat. no.1089; Medicult) was put in the middle and was surrounded by eight microdroplets of HEPES EBSS under oil. The oocytes were put individually in each droplet. Approximately 2 µL from the bottom of

the Tea tube was added to the PVP droplet and checked under the microscope. If no sperm were found, a microdroplet from the resuspended pellet itself was put near the PVP .The microinjection procedure was done using an inverted-phase microscope (Nikon diaphot TMD; Nikon, Tokyo, Japan) with a heated stage (Swemed, Frolunda, Sweden) and equipped with Hoffman condenser (Modulation Optics Inc. Greenvale, NY) and micromanipulation set (Narishige Inc. Tokyo, Japan). The procedure was allowed to be followed on a monitor (model no. KX-14CPI, Sony, Tokyo, Japan) connected to a video camera (Panasonic color CC TV camera model no. WVCL 300/6; Matsushita Communication Industrial Co., Tokyo, Japan) that was attached to the microscope. The injecting micropipettes were ready made (Humagen Fertility Diagnostics, Charlottesville, V A) and the holding pipettes were from Cook (cat. no. K-HPIP-1000; Cook, Eight Mile Plains, Queensland, Australia). The microinjection pipette was lowered in the PVP microdroplet and one sperm was chosen (the best available motile and morphologically well-formed sperm) and was immobilized by touching its tail near the midpiece with the injecting micropipet. The immobilized sperm was aspirated, tail first, into the injecting pipette. After securing the oocyte in position with the holding pipette (polar body at 6 or 12 o'clock position), the injecting pipette was introduced at the 3 o'clock position through the zona pellucida, oolemma, and deeply into the cytoplasm. The sperm then was injected slowly and the pipette was withdrawn. No attempt was made to aspirate the cytoplasm. After injection, the oocytes were rinsed and incubated under oil in Ham's F-10 and 10% patient serum. The next morning, the oocytes were examined for evidence of fertilization and transferred to fresh media (Ham's F-10 and 20% patient serum). The rate of cleavage and morphological appearance of embryos were observed. Embryo transfer was done on day 2 after the pick-up, using the Wallace catheter (cat. no. 1816N; H.G. Wallace Limited, Cholchester, United Kingdom) or the Labotect catheter (Labotect, Bovender-Gottingen, Germany) if the Wallace catheter could not be introduced. Luteal phase

support was given routinely in the form of 2,500 IU hCG every 4th day. Cases that were considered at high risk of developing ovarian hyperstimulation syndrome were given a daily P injection (100 mg, P USP; STERIS, Phoenix, AZ). A serum β-hCG test was done 2 weeks after ET to diagnose pregnancy, and an ultrasound examination was done after 2 to 3 more weeks for patients with a positive test. Clinical pregnancy was diagnosed by the presence of a gestational sac.

RESULTS

A total of 23 patients with infertility due to obstructive azoospermia underwent 24 cycles of microsurgical sperm retrieval in combination with ICSI. The total number of metaphase II oocytes injected was 226 and normal fertilization (two pronuclei [2PN]) was evident in 141 oocytes (62%). All patients reached the ET stage with an average of 3.7 embryos per transfer. Ten clinical pregnancies resulted in this series, achieving a clinical pregnancy rate of 42%. Three healthy babies were born, one abortion occurred in the first trimester, and the rest of the pregnancies are ongoing.

Tables 3 and 4 summarize the results of microepididymal sperm aspiration and testicular sperm aspiration cases respectively. Table 5 illustrates a comparison between microepididymal sperm aspiration and testicular sperm aspiration results. There was no significant difference in the fertilization and pregnancy rates between both groups ($P > 0.05$).

In 17 cycles, the spermatozoa were aspirated microsurgically from the epididymis. The epididymal samples aspirated contained large numbers of spermatozoa in most of the cases, ranging between 0.3 and 200 X 10^6/mL, with an average of 30 X 10^6/mL. The initial sperm motility ranged between 1% and 25% with an average of 6% and forward grade 1 and 2 (World Health Organization standards) (12). The abnormal forms ranged from 65% to 100%

according to strict criteria (13). The abnormality was mostly in the midpiece because of the presence of a cytoplasmic droplet whereas the morphology of the sperm head was mostly normal. In seven cases the epididymis was either absent or completely fibrosed and testicular biopsies were taken. The sperm retrieved ranged from very few spermatozoa to 2 X 10^6/mL, with an average of 5% motility and forward grade 1 + or barely twitching in place.

Cryopreservation was done for 45 2PN oocytes in eight cycles. Three pregnant patients have a total of 22 cryopreserved embryos for future use. All patients had the rest of the aspirated epididymal and testicular spermatozoa cryopreserved. Cryothawed epididymal spermatozoa was used in one case and resulted in successful fertilization and an ongoing pregnancy.

DISCUSSION

Men with surgically unreconstructable obstructive azoospermia are relatively common among infertile patients. In Egypt, congenital absence of the vas deferens accounts for $\geq$ 10% of the cases of obstruction (14). Retroperitoneal vasal obstruction, multiple obstructions, and failed attempts of epididymovasostomy are other common examples of unreconstructable causes of infertility (15). Using epididymal or testicular sperm in patients with obstructive azoospermia to fertilize their wive's oocytes in vitro is a great achievement and a highly valued procedure especially in countries, like ours, where no sperm donation is allowed. The procedure is not easy and requires a collaboration between an experienced IVF team and a urologic or andrological microsurgeon.

Recently, the new therapy involving the surgical retrieval of sperm combined with assisted conception has given new hope to those previously considered "sterile" males. Pryor et al. (1), in 1984, first reported pregnancy with the use of surgically aspirated sperm for IVF. They aspirated spermatozoa from the vas deferens of a man after vasovasostomy failed to

correct a tuberculous obstruction of the vas. In 1985, Temple-Smith et al. (2) reported a pregnancy after IVF using microsurgically aspirated sperm from the epididymis. In 1988, Silber et al. (3) reported pregnancies using microsurgically aspirated spermatozoa from the epididymis of men who were azoospermic because of congenital absence of the vasa deferentia. Microsurgical epididymal sperm aspiration was tried with conventional IVF, partial zona dissection, and sub zonal sperm injection with overall fertilization and pregnancy rates of 19% and 27.5%, respectively (5). Combined microepididymal sperm aspiration and ICSI proved to be a highly successful approach, achieving fertilization and pregnancy rates of 58% and 35.7%, respectively (7). When the epididymis was absent or fibrosed, no spermatozoa could be retrieved and testicular biopsies were taken to extract spermatozoa. Successful fertilization and pregnancies have been reported using testicular sperm for ICSI (9). Because of the consistently very good results using epididymal and testicular spermatozoa with ICSI in comparison to conventional IVF, ICSI may be mandatory for all patients with congenital absence of the vas deferens or with irreparable obstructive azoospermia (8). The present study confirmed that ICSI, using micro surgically retrieved epididymal or testicular sperm, is a highly successful technique, achieving fertilization and pregnancy rates of 62% and 42%, respectively.

Maturational changes occur during the transport of the spermatozoa in the epididymis. Sperm are immotile or ineffectively motile at the time they enter the caput. However, the fertilizing capacity of the sperm aspirated from the caput that had not traversed the complete epididymis was confirmed by Silber et al. (3) when they succeeded in obtaining a pregnancy from sperm aspirated from the caput in a case of congenital absent vas. Based on the current understanding of sperm maturation, Jow et al. (16), in 1993, suggested that sperm may acquire motility in an obstructed system through prolonged confinement within the reproductive tract, direct contact with refluxed epididymal factors, and retrograde migration

of sperm after contact with epididymal environment. Furthermore, chronic obstruction also may cause adaptation of the testicular epithelium, which allows acquisition of intratesticular sperm motility and maturation of fertilizing capacity to occur. It is plausible that a combination of these factors would indeed be necessary to account for these observations.

Epididymal samples usually have a high count but very low motility; in this study the count ranged between 0.3 and 200 X 10^6/mL with an average of 30 X 10^6/mL and the motility had an average of 6% forward grade 1 and 2. Such semen characteristics usually are associated with poor fertilization in conventional IVF and the use of micromanipulation improved the chances of fertilization of these spermatozoa with impaired ability (5). The most precise and efficient form of micromanipulation is ICSI. The number and the motility of sperm does not affect the success rate in terms of fertilization or pregnancy with the use of ICSI as shown in this study and as reported previously (17, 18).

Marmar et al. (19), in 1993, reported that a mobile program, in which sperm collection and egg retrieval performed in two centers away from each other, is feasible. In their study, they were able to transfer processed semen to other centers; the farthest shipment was 90 miles with no impact on sperm survival. In the present study we transferred sperms aspirated in HEPES media directly to the IVF center without processing or incubation; the transport time ranged from 0.5 to 1 hour depending on the traffic. This success with specimen transport may encourage other centers that do not have urologic surgical services within an IVF unit to pursue offering this treatment.

In this study, a good fertilization rate was achieved in the absence of cytoplasmic aspiration at the time of sperm injection. This methodological aspect is different from that of many other ICSI investigators who consider cytoplasmic aspiration to be essential to good fertilization. It is our routine policy in all ICSI cases, not to aspirate the cytoplasm (18). The injection needle is introduced deeply into the cytoplasm and piercing of the oolemmal

membrane usually occurs when the tip of the needle is near the 9 o'clock position. Piercing of the oolemmal membrane is assured when a yield of the pulled membrane is felt and the sperm is released away from the tip of the pipette without increasing the pressure of the injection. A good fertilization rate of 62% using this technique indicates that aspirating the cytoplasm is not essential for activating the oocyte.

Sperm processing used in our ICSI cases is also different from that of other ICSI investigators, as we never used Percoll gradient and we use only a Tea tube for separating motile spermatozoa. Spermatozoa of testicular and epididymal origin usually have a very slow forward grade and sometimes only shake in place. In most of these cases no motile spermatozoa are expected to be separated using the Tea tube, and a microdroplet from the resuspended pellet itself is used to search for a motile sperm using the injecting pipette.

This sperm processing method without the use of Percoll may prove to have an interesting implication for embryo cleavage, quality, implantation, and normality of infants born when compared with other ICSI programs. This experience may clarify the different outcomes in studies that use a sperm selection by Percoll in which silica gel particles may have been injected into the egg during ICSI.

In conclusion, we reported our first 24 cycles of ICSI using testicular and epididymal sperm. It is a highly successful technique in cases of obstructive azoospermia. Fertilization rate was 62% and 10 clinical pregnancies resulted.

Table 1 Microepididymal Sperm Aspiration Characteristics, Etiology of Obstructive Azoospermia and Operative Findings

Patient no.	Husband's age	Wife's age	Infertility period	Etiology of obstructive azoospermia	Operative findings
	y	y	y		
1	35	33	6	Bilateral congenital absence of vas deferens (CAVD)	Mild adhesions, right epididymis full
2	40	35	12	Right CAVD, Left absent testis	Right massive adhesions, thick epididymal capsule
3	39	35	9	Left CAVD, multiple obstructions in the right vas	Left massive adhesions, thick epididymal capsule
4	35	35	4	Bilateral CAVD, previous implantation of alloplastic spermatocele on the right side	Epididymal head and body were present, minimal adhesions
5	38	31	12	Right CAVD with collapsed right epididymis, acquired left obstruction	Left vas totally obstructed, moderate adhesions aspiration from epididymal head
6	43	36	12	Bilateral acquired multiple obstructions	Dense adhesions, multiple obstructions.
7	34	34	12	Bilateral CAVD	Epididymal head and body was present, mild adhesions
8	45	35	14	Bilateral CAVD	Massive adhesions from previous surgery, thick epididymal capsule
9	32	31	9	Acquired multiple obstructions	Left massive adhesions thick and vascular epididymal capsule, aspiration was tried at two different sites in the head
10	38	33	8	Acquired multiple obstructions	Massive adhesions, multiple obstructions
11	36	30	8	Right CAVD, left acquired obstruction	Massive adhesions
12	52	38	18	Bilateral CAVD	No adhesions, puncture of the epididymal head
13	38	31	5	Atrophic right testis after orchitis, acquired left multiple obstructions	Marked adhesions, loculated hydrocele
14	45	37	15	Acquired bilateral obstructions, failed right epididymovasostomy	Moderate adhesions, epididymal head was full
15	45	33	14	Bilateral CAVD	No adhesions, epididymal head was full
16	43	34	12	Bilateral CAVD	No adhesions

Table 2 Testicular Sperm Aspiration Patient Characteristics and Etiology of Obstruction

Patient no.	Husband's age	Wife's age	Infertility period	Etiology of obstructive azoospermia	Operative findings
	y	y	y		
1	41	28	7	Single testicle, acquired epididymal obstruction	Epididymis completely fibrosed
2	40	31	10	Acquired obstruction, failed right epididymovasostomy	Extenstive fibrosis, empty epididymis
3	43	38	13	Acquired obstruction	Completely fibrosed epididymis (bilateral)
4	28	21	7	Acquired obstruction, previous bilateral epididymovasostomy	Massive adhesions, empty epididymis
5	49	36	9	Vasectomy for contraception, failed reconstructive surgery twice	Massive adhesions, no epididymal tissues
6	43	40	10	Right orchidopexy operated upon at 16 years; partial obstruction. At day of ICSI; semen AZO; testicular sperm aspiration was done to obtain spermatozoa	Massive adhesions, very vascular empty epididymis
7	32	28	5	Bilateral congenital absence of vas deferens	Total absence of the epididymis

Table 3 Outcome of ICSI Using Epididymal Sperm

Patient no.	Characteristics of the aspirated semen: Count	Forward grade 1	2	3	4	Normal forms % (Kruger's criteria)	No. of oocytes injected (MII)	No. of fertilized oocytes (2PN)	No. of frozen zygotes	No. of embryos transferred	Pregnancy
	×10⁶	%				%					
1	200	5	—	—	—	10	8	4	—	4	—
2	5	2	—	—	—	5	3	3	—	2	—
3	15	15	—	—	—	0	20	12	7	5	—
4	2.5	5	—	—	—	20	3	2	—	2	Chemical
5	30	15	—	—	—	15	15	10	5	5	Yes (Twins)
6	40	1	—	—	—	10	3	2	—	2	—
7	80	2	—	—	—	10	7	1	—	1	—
8	5	1	—	—	—	25	1	1	—	1	Yes
9	100	10	5	—	—	5	22	17	12	4	Yes (Triplet)
10	20	10	—	—	—	35	15	7	—	5	—
11	15	15	15	—	—	0	13	7	2	5	—
2 (repeat cycle)	10	1	—	—	—	5	4	3	—	3	Yes
12	10	1	—	—	—	0	5	4	—	3	Yes
13	3	1	—	—	—	10	16	10	5	5	Yes
14	10	5	—	—	—	0	12	8	3	5	—
15	5	1	—	—	—	0	17	12	6	5	—
16	0.3	1	—	—	—	10	8	6	—	6	—

Table 4 Outcome of ICSI Using Testicular Sperm

Patient no.	Testicular sperm retrieved: Count	Forward grade 1	2	3	4	Normal forms	No. of oocytes injected (MII)	No. of fertilized oocytes (2PN)	Frozen zygotes	No. of embryos transferred	Pregnancy
	×10⁶	%				%					
1	1	1	—	—	—	10	5	3	—	3	Yes
2	0.2	5	—	—	—	5	9	5	—	5	Yes
3	0.05	—	—	—	—	10	4	1	—	1	Chemical
4	0.5	10	—	—	—	0	6	4	—	4	Chemical
5	1	5	—	—	—	0	17	11	5	5	Yes
6	0.5	1	—	—	—	1	3	2	—	2	—
7	1	1	—	—	—	0	10	6	—	5	Yes

Table 5 Results of Microepididymal and Testicular Sperm Aspiration*

	No. of cycles	No. of MII oocytes injected	No. of 2PN oocytes	No. of cryopreserved embryos	No of embryos transferred	Clinical pregnancies
Microepididymal sperm aspiration	17	172	109 (63)†	40	63	6 (35)
Testicular sperm aspiration	7	54	32 (59)	5	25	4 (57)
Total	24	226	141 (62)	45	88	10 (42)

* Values in parentheses are percentages. †$P < 0.05$.

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Chapter 8

Successful intracytoplasmic sperm injection without performing cytoplasmic aspiration

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Abstract

Objective: To compare the effect of cytoplasmic aspiration versus no aspiration before intracytoplasmic sperm injection (ICSI) on the rate of oocyte damage, fertilization rate, and embryo quality.

Design: A randomized prospective study on sibling oocytes.

Setting: The Egyptian IVF-ET Center, Cairo, Egypt.

Participants: Fifty-eight patients who were infertile due to male factor who underwent 60 ICSI cycles.

Intervention: Intracytoplasmic sperm injection was performed on randomly allocated metaphase II oocytes with cytoplasmic aspiration in group I and without cytoplasmic aspiration in group II before sperm injection.

Main Outcome Measure: Fertilization rate, oocyte damage rate, and embryo quality.

Results: Normal fertilization rate per injected oocyte was 61.4% in group I compared with 62.5% in group II. The damage rate per injected oocyte was 16.8% in group I as compared with 4.6% in group II. Grade I embryos were 24.5% in group I compared with 48.5% in group II.

Conclusion: Cytoplasmic aspiration before sperm injection in ICSI is not essential for oocyte activation. It did not improve the rate of normal fertilization. On the other hand, it increased the damaged oocyte rate and the rate of cytoplasmic fragments. Fertil Steril® 1996;66:256-9

The development of intracytoplasmic sperm injection (ICSI) technique in humans was reported first by Lanzendorf et al. (1) in 1988. In their work, they proved that the human oocyte was capable of surviving the injection and supporting the formation of male and female pronuclei. The first four human pregnancies using ICSI that were reported first by Palermo et al. (2) in 1992 was a breakthrough in the field of assisted fertilization. High fertilization and pregnancy rates, comparable to those reported in nonmale factor patients, were achieved in patients with severe male factor infertility (3) and obstructive azoospermia (4) with the use of ICSI. Therefore, most IVF centers, including our own, have shifted to the use of ICSI technique as the only method for assisted fertilization (5).

Cytoplasmic aspiration was considered an integral part of the ICSI procedure and an essential step for activation of the oocyte (6). It even was reported recently that vigorous aspiration of the oocyte cytoplasm is a crucial factor for the success of ICSI (7). On the contrary, in our ICSI program, the injection always has been done routinely without cytoplasmic aspiration, and we have achieved high fertilization and clinical pregnancy rates. The aim of the present study was to compare ICSI, with and without performing cytoplasmic aspiration, on sibling oocytes to see the outcome on the rate of oocyte damage, normal fertilization, and quality of embryos.

MATERIALS AND METHODS

Patients

Fifty-eight patients who underwent 60 ICSI cycles at the Egyptian IVF-ET center are included in this study. The mean age of the female partners was 32.4 ± 4.2 years and the mean duration of infertility was 8.5 ± 2.5 years. All patients had male factor infertility. The initial semen evaluation was done on at least two occasions before the treatment cycle. They had a sperm count < 10 X 10^6/mL, motility < 30%, and abnormal forms > 60%. Ovulation induction was done using GnRH agonist analogue, long protocol, and hMG (8). Oocyte pick-up was done 36 hours after hCG injection (9) through ultrasonic transvaginal route. Semen processing and details of ICSI was described previously (5).

Microinjection Procedure

After securing the oocyte in position with the holding pipette (polar body at 6 or 12 o'clock position), the injection pipette was introduced at 3 o'clock position through the zona pellucida, oolemma, and deeply into the cytoplasm. In group I, a negative pressure was applied until the cytoplasm was withdrawn inside the injecting pipette approximately one quarter of the diameter of the oocyte then the sperm was injected slowly and the pipette was withdrawn. In group II, no attempt was done to aspirate the cytoplasm. Piercing of the oolemmal membrane was assured when a yield of the pulled membrane at the periphery of the oocyte was felt and the sperm was released away from the tip of the pipette without increasing the pressure of the injection. If the pressure inside the injecting pipette was low at the moment of piercing the oolemma, the spermatozoon and minimal cytoplasm may flow into the tip of the pipette. After injection, the oocytes were rinsed and incubated under oil in Ham's F-10 (GIBCO Laboratory, Grand Island, NY) supplemented with 10% patient serum.

The next morning, the oocytes were examined for evidence of fertilization and transferred to fresh media (Ham's F-10 supplemented with 20% patient serum). Assessment of the embryos was performed by adopting a modified morphological criteria classification (10) as follows: grade 1, good quality, blastomeres uniform in size and shape, ooplasm with no granularity, no anucleate fragments; grade 2, intermediate quality, blastomeres uneven in size and shape, ooplasm slightly granular, anucleate fragments affecting <25% of blastomeres; and grade 3, poor quality blastomeres uneven in size and shape, ooplasm extremely granular, anucleate fragments affecting >25% of blastomeres.

Embryo transfer was done on day 2 after the pickup with the best morphologically looking embryos from both groups. Luteal phase support was given routinely in the form of 2,500 IU of hCG every 4th day. Patients that were considered at high risk of developing ovarian hyperstimulation syndrome were given daily P injection. Serum β-hCG test was done 2 weeks after ET to diagnose pregnancy, and an ultrasonic examination was done after 2 to 3 more weeks for patients with a positive test. Clinical pregnancy was diagnosed by the presence of a gestational sac with fetal echoes. Statistical analysis was done using X^2 test.

RESULTS

Fifty-eight patients underwent 60 ICSI cycles. A total of 580 oocytes were retrieved, 460 oocytes were at metaphase II (79%). In group I (cytoplasmic aspiration), of 220 oocytes injected, 37 oocytes were damaged (16.8%) and 135 oocytes (61.4%) showed normal (two pronuclei) fertilization. In group II (no cytoplasmic aspiration), out of 240 oocytes injected 11 oocytes were damaged (4.6%) and 150 oocytes (62.5%) showed normal fertilization (Table 1). The rate of damaged oocytes was significantly higher in group I as compared with group II ($X^2 = 18.38$, $p = 0.001$). However, there was no statistically significant difference in the fertilization rate between the two groups ($X^2 = 0.06$, $p = 0.802$).

The number and quality of embryos in each group is shown in Table 2. There was a statistically significant higher percentage of grade I embryos in group II; the rate of grade III embryos was significantly higher in group I ($X^2 = 17.08$, $p = 0.001$).

Seventy-five embryos were cryopreserved from both groups. Clinical pregnancy was diagnosed in 21 patients (35%), including 17 singletons and 4 twins.

DISCUSSION

In this study, we compared the ICSI procedure with cytoplasmic aspiration before sperm injection versus no aspiration on sibling oocytes. There was no statistically significant difference in the fertilization rate between the two groups. These results would question the hypothesis of the importance of cytoplasmic aspiration in inducing oocyte activation. The high fertilization and cleavage rate in the oocytes injected without cytoplasmic aspiration indicate that there are other factors that induce oocyte activation. Puncturing the oocyte cytoplasm with a pipette for injection in itself was considered a cause for oocyte activation

(11). Edwards and Van Steirteghem (12) suggested that the calcium contained in the medium with which the sperm is injected into the oocyte may be the impulse initiating oocyte activation by triggering calcium-induced calcium release, a mechanism known to work in some species. Hoshi et al. (13) used calcium ionophore because they thought that increased Ca^{2+} in the ooplasm has a positive effect on oocyte activation. Electroporation also has been used to accelerate the flow of Ca^{2+} into the ooplasm (11, 14). Tesarik et al. (15) reported that the activation of human oocytes after ICSI does not occur as a result of the injection procedure, and they stated that elevated calcium concentrations in the medium in which ICSI is performed is not needed and even may be an overload. In their experiment, they concluded that activation of the oocyte occurs as a result of the release of sperm-activating factor. In a recent study by the same author (7), it was suggested that vigorous aspiration of the oocyte cytoplasm may facilitate fertilization after ICSI by increasing the oocyte Ca^{2+} load at the time of injection, by establishing a more intimate contact of the injected sperm head with oocyte intracellular Ca^{2+} stores or by a conjunction of these mechanisms. Cytoplasmic aspiration also was considered an essential step for oocyte activation by other investigators (6). Our results clearly demonstrated that cytoplasmic aspiration is not at all an essential factor for oocyte activation. More likely, the oocyte is activated from the release of sperm factors as reported by Dale and De Felice in 1990 (16). Fishel et al. (17) demonstrated a highly significant increase in fertilization rate in ICSI when sperm tail plasma membrane was damaged. They suggested that damaging the sperm plasma membrane release a sperm cytosolic component for egg activation.

Our results suggest that cytoplasmic aspiration might be a way of making sure that the oolemmal membrane is pierced rather than a factor in oocyte activation. Until recently, the detailed methodology of penetration into the ooplasm in performing ICSI was not described clearly in the literature. Palermo et al. (6) reported that the oolemma may be particularly soft

and elastic depending upon the time spent by the oocyte in vivo or in vitro, rendering it difficult to penetrate. We believe that one of the main reasons for the low fertilization rate in ICSI by some investigators in early reports was probably that the oolemmal membrane was not perforated in all oocytes and that the sperm was deposited outside the cytoplasm. Therefore, the function of cytoplasmic aspiration is to ensure that the oolemmal membrane is pierced rather than its role for oocyte activation. Fishel et al. (17) clearly described their technique of piercing the oolemma. They stated that the oolemma often invaginated and created an artificial furrow in the ooplasm, simulating penetration of the oolemma. In most instances they had to manipulate the oolemma either by tiny sharp thrusts of the microneedle once the latter had traversed >75% of the diameter of the oocyte or to aspirate the oolemma (which is very stretchable) into the microneedle or a combination of both. We always have been able to pierce the oolemmal membrane without any attempt of cytoplasmic aspiration. Piercing of the oolemmal membrane was confirmed when a yield of the pulled membrane was felt above and below the introduced injection pipette accompanied with a release of the sperm away from the tip of the injection pipette without increasing the injection pressure. If the pressure inside the injecting pipette was low, at the moment of piercing the oolemma, the spermatozoon and minimal cytoplasm may flow into the tip of the pipette.

The rate of oocyte damage in our study was significantly higher in group I (16.8%) as compared with group II (4.6%), indicating that cytoplasmic aspiration increases the rate of oocyte damage at least in our hands. Palermo et al. (6) reported that the mechanical damage from the injection procedure was 7.1% as compared with higher rates of damage reported in earlier studies. They suggested that the reduction of oocyte damage rate was most likely a result of avoiding the area of polar granularity during insertion of the injection pipette, therefore avoiding the place where possibly the germinal vesicle breakdown takes place and where the chromosomes and mitotic spindles are located. Fishel et al. (17) demonstrated a

highly significant decrease in cytoplasmic fragmentation when a permanently immobilized spermatozoon is utilized for ICSI, suggesting that immobilization might prevent disruption the ooplasm by an active sperm tail. The technique of cytoplasmic aspiration before sperm injection may produce mechanical disruption of the cytoplasmic structures, including the spindle. Moreover, the process of cytoplasmic aspiration might result in an increased volume of the fluid injected inside the cytoplasm during the deposition of the sperm. Nevertheless, it should be mentioned that the higher incidence of oocyte damage observed in this study with cytoplasmic aspiration compared with injection only may have been due to the fact that the former technique is not the routine technique in our lab.

The results of this work has shown a statistically significant higher percentage of grade I embryos in the group with no cytoplasmic aspiration as compared with the group with cytoplasmic aspiration. The implantation rate of embryos resulting from each technique was not evaluated because ET was done using a mixture of the best quality embryos from both groups.

Using this technique of no aspiration of the cytoplasm as a routine in our ICSI program, we have achieved a fertilization rate of 61% and a clinical pregnancy rate of 35% in a series of 350 ICSI cases (18). In two other reports using the same technique, similar fertilization and pregnancy rates were achieved (5, 19). To the best of our knowledge, this is the first report in the literature that demonstrates that cytoplasmic aspiration in ICSI procedure is not essential for oocyte activation. High fertilization rate and high quality embryos were achieved without cytoplasmic aspiration.

Table 1 Fertilization and Damaged Oocyte Rates

	Group I cytoplasmic aspiration	Group II no cytoplasmic aspiration
No. of injected oocytes (metaphase II)	220	240
No. of damaged oocytes*	37 (16.8)	11 (4.6)†
No. of two pronuclear oocytes	135 (61.4)	150 (62.5)‡

* Values in parentheses are percentages.
† $\chi^2 = 18.38$, $P = 0.001$.
‡ $\chi^2 = 0.06$, $P = 0.802$.

Table 2 Cleavage and Embryo Quality

	Group I cytoplasmic aspiration	Group II no cytoplasmic aspiration
No. of two pronuclear oocytes	135	150
No. of cryopreserved embryos	33	42
Oocytes arrested at two pronuclear stage	8	5
No. of cleaved embryos*	94 (92)	103 (95)
Grading*†		
Grade I	23 (24.5)	50 (48.5)
Grade II	34 (36.2)	36 (35)
Grade III	37 (39.3)	17 (16.5)

* Values in parentheses are percentages.
† $\chi^2 = 17.08$, $P = 0.001$.

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Chapter 9

Intracytoplasmic sperm injection in obstructive and non-obstructive azoospermia

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Abstract

We compared the results of intracytoplasmic sperm injection (ICSI) in: (i) obstructive versus non-obstructive azoospermia, (ii) obstructive azoospermia using epididymal versus testicular spermatozoa and (iii) acquired versus congenital obstructive azoospermia due to congenital absence of the vas deferens (CAVD). A retrospective analysis was done of 241 consecutive ICSI cycles done in 103 patients with non-obstructive azoospermia and 119 patients with obstructive azoospermia. In the obstructive group, 135 ICSI cycles were performed. Epididymal spermatozoa were used in 44 cycles and testicular spermatozoa in 91 cycles. In the non-obstructive group, 106 cycles were performed. The fertilization and pregnancy per cycle rates were 59.5 and 27.3% respectively using epididymal spermatozoa, 54.4 and 31.9% respectively using testicular spermatozoa in obstructive cases, and 39 and 11.3% respectively in non-obstructive cases. The fertilization and pregnancy per cycle rates were 56.6 and 37% respectively in acquired obstructive cases, and 55.2 and 20.4% respectively in CAVD. In conclusion, ICSI using spermatozoa from patients with acquired obstructive azoospermia resulted in significantly higher fertilization and pregnancy rates as compared to CAVD and non-obstructive cases.

INTRODUCTION

Surgically unreconstructable obstruction of the male genital tract is a relatively common aetiology of azoospermia among infertile men (Girgis et al., 1969). Patients with obstructive azoospermia due to congenital absence of the vas deferens (CAVD), or those who had suffered failure of reconstructive surgery, have historically been considered hopelessly infertile. Recently, new therapy involving surgical retrieval of spermatozoa combined with assisted reproduction has given new hope to those patients previously considered 'sterile' males. The reported fertilization and pregnancy rates after the combination of microsurgical epididymal spermatozoa aspiration (MESA) and standard in-vitro fertilization (IVF) are poor, not exceeding 20 and 11% respectively (Pryor et al., 1984; Temple-Smith et al., 1985; Silber et al., 1988, 1990; Schlegel et al., 1994).

The introduction of intracytoplasmic sperm injection (ICSI; Palermo et al., 1992) has improved the results significantly, and high fertilization and pregnancy rates have been reported after the combination of MESA and ICSI (Silber et al., 1994; Tournaye et al., 1994; Hovatta et al., 1995; Mansour et al., 1996a).

In some patients with obstructive azoospermia, MESA fails to retrieve any spermatozoa, either due to fibrosis or complete absence of the epididymis. Because neither sperm motility nor morphology significantly influences the results of ICSI (Van Steirteghem et al., 1993; Mansour et al., 1995), it seems a logical approach to use testicular spermatozoa in cases when it is impossible to retrieve any spermatozoa from the ejaculate or the epididymis. Until recently, the fertilizing capacity of testicular spermatozoa was unexplored. It was reported in 1993 that the use of testicular spermatozoa in ICSI for cases of obstructive azoospermia could achieve fertilization and pregnancy (Craft et al., 1993; Schoysman et al., 1993a,b). Following that, different programmes reported the use of testicular spermatozoa with ICSI (Devroey et

al., 1994; Bourne et al., 1995; Craft and Tsirigotis, 1995; Nagy et al., 1995; Silber et al., 1995; Fahmy et al., 1996; Mansour et al., 1996a,b). Recently, the indications for testicular sperm extraction (TESE) and ICSI were expanded to include cases with non-obstructive azoospermia due to severe impairment of spermatogenesis (Devroey et al., 1995; Gil-Salom et al., 1995; Tournaye et al., 1995; Kahraman et al., 1996a,b; Silber et al., 1996; Tournaye et al., 1997).

Different investigators have studied fertilization and pregnancy rates in ICSI, using epididymal and testicular spermatozoa (Hovatta et al., 1995; Nagy et al., 1995; Silber et al., 1995; Fahmy et al., 1996), for patients with obstructive and non-obstructive azoospermia (Devroey et al., 1996; Kahraman et al., 1996) and congenital and acquired obstructive azoospermia (Chen et al., 1995).

The aim of this work was to compare the results of ICSI for (i) obstructive versus non-obstructive azoospermia, (ii) acquired obstructive azoospermia versus congenital obstruction due to CAVD, (iii) epididymal versus testicular spermatozoa in obstructive azoospermia.

MATERIALS AND METHODS

This retrospective analysis involved 241 consecutive ICSI cycles for azoospermic patients that were performed between September 1994 and September 1996. The routine andrological work-up of the male patients included conventional semen analysis, endocrinological profile, culture of urine and examination of expressed prostatic secretion. Other investigations, such as transrectal ultrasound and preoperative diagnostic testis biopsy, were performed whenever indicated. Karyotyping to exclude chromosomal abnormalities was routinely done for patients with very small testes (<3-5 cm). Patients with Klinefelter's syndrome were excluded

from the study. Counseling was done for each of the couples with CAVD, informing them about the possibility of having an offspring with cystic fibrosis (CF). They were told that if the wife is CF negative then the risk of having an offspring with CF is $\simeq$ 0.3% (Patrizio et al., 1993).

All patients were counseled and signed a consent approved by our internal ethical committee. Ovarian stimulation was induced using a long protocol of gonadotrophin-releasing hormone agonist and human menopausal gonadotrophin (Aboulghar et al., 1994). Human chorionic gonadotrophin (HCG) was given (10 000 IU i.m.) when three or more follicles reached a mean diameter of $\geq$ 18 mm. Ovum retrieval was scheduled 36 h after HCG injection. Oocyte handling and details of microinjection have been described previously (Mansour et al., 1995).

Sperm retrieval

MESA was performed under general anaesthesia, as an outpatient procedure, as described before (Mansour et al., 1996a,b). Percutaneous sperm aspiration (PESA) was successfully performed in 25 patients (18 with CAVD and seven with acquired obstruction), using a 25-gauge butterfly needle filled with tissue culture medium, connected to a 1 ml syringe under local infiltration anaesthesia (Craft et al., 1995). We attempted PESA in nine more cases, but the epididymal aspirates either failed or showed no motile spermatozoa, and TESE was done instead. Testicular biopsy was done under local anaesthesia, using a technique of testicular biopsy processing described previously (Fahmy et al., 1996). In some cases of non-obstructive azoospermia, an extensive search for spermatozoa was performed (up to 4 h) and none was found. Then spermatids were looked for and used for injection. Oval spermatids looked like the head of a spermatozoon with a dark elongated nucleus and a lighter area in the middle. Round spermatids were identified by their size, which is smaller than all other spermatogenic cells, and also by the shape of their nuclei, which are dark, rounded structures

with a lighter area in the centre; they have a minimal cytoplasm, and a cytoplasmic membrane that can be identified easily. One of the criteria used to identify round spermatids was the spinning of the round spermatid at the tip of the pipette during aspiration, which has been described by Tesarik and Mendoza (1996). Normal size microinjection pipettes (inner diameter 5-7 μm) were used to inject the elongated spermatids, and larger diameter pipettes (8-10 μm) were used to inject the round spermatids. Trials of hitting the spermatid with the injection pipette were done in an attempt to break the cytoplasmic membrane.

Statistical analysis

The significance of differences in frequencies between groups were compared by the X^2 test. Differences between means were tested by analysis of variance. All statistical tests were assessed at the 5% level of significance.

RESULTS

A total of 222 azoospermic patients underwent 241 consecutive ICSI cycles; 119 patients with obstructive azoospermia underwent 135 cycles. The spermatozoa were retrieved from the epididymis in 44 cycles (MESA, n = 18; PESA, n = 22; cryothawed MESA, n = 4). TESE was done for 81 patients, who underwent 91 ICSI cycles (five cycles using cryothawed testicular spermatozoa). The aetiology of infertility and indications for surgical retrieval of spermatozoa are summarized in Table I.

Non-obstructive azoospermia due to severe impairment of spermatogenesis was observed in 103 patients who underwent 106 cycles of ICSI and TESE. According to previous histopathological testicular biopsy reports, maturation arrest was diagnosed in 58 cycles. Partial or incomplete arrest, with some testicular tubules showing late spermatids, was

diagnosed in 28 cycles. Nine cycles were diagnosed as complete arrest at primary spermatocytes. Twenty one cycles were diagnosed as complete arrest at early spermatids. Sertoli cell-only syndrome (SCO), where all the tubules were devoid of any spermatogenic cells, was diagnosed in 17 cycles. Mixed SCO, where some tubules showed some spermatogenic cells at various stages, was diagnosed in 25 cycles. In five patients, the histological finding of the previous biopsy showed generalized proportionate reduction of all spermatogenic cells (hypospermatogenesis). This is different from partial spermatogenic arrest, where there is a reduction of the number of late spermatids, while the numbers of other spermatogenic cells are normal. In one patient, the testicular biopsy report showed complete tubular hyalinization. It is worth mentioning that this particular patient had lepromatous leprosy. Table II illustrates the patients' characteristics in the obstructive and non-obstructive groups.

The overall fertilization rate was 50.1%, and 213 (88.4%) cycles reached the embryo transfer stage. Clinical pregnancy was achieved in 53 cases, with a clinical pregnancy rate of 25% per transfer and 22% per cycle. In the obstructive group, 135 cycles were performed, the fertilization rate was 56% and all cycles reached the embryo transfer stage. Clinical pregnancy was diagnosed in 41 cycles, achieving a clinical pregnancy rate per cycle of 30.4% (Table III).

Comparing the use of testicular and epididymal spermatozoa in the obstructive group, the fertilization rate and pregnancy rate per cycle were respectively 59.5 and 27.3% in the epididymal group and 54.4 and 31.9% in the testicular group. There was no statistically significant difference between the two groups.

When the obstructive group was re-analyzed according to the cause of obstruction, the fertilization and pregnancy rates were respectively 56.6 and 37% in the acquired obstruction group and 55.2 and 20.4% in the group with CAVD (Table IV). The fertilization rate was

similar in both groups, but the pregnancy rate was significantly higher in the acquired obstructive group ($P < 0.05$).

In the non-obstructive group, ICSI was performed in 106 cycles, and 79 cycles reached the embryo transfer stage (74.5%). The fertilization rate was 39% and 12 clinical pregnancies were diagnosed, achieving a clinical pregnancy rate of 15.2% per transfer and 11.3% per cycle (Table III). The results of the non-obstructive group were re-analysed according to the type of spermatogenic cell used for injection (Table V). Spermatozoa could be found in the testicular biopsies in 60 cycles (56.6%) and used for ICSI. The fertilization and pregnancy rates for these cycles were 39.7 and 18.3%. In 11 cycles (10.4%), the number of normal live spermatozoa was too few to inject all the retrieved oocytes, and spermatids were also injected, with a fertilization rate of 39% but no pregnancy resulted. In 15 cycles (14.2%), no spermatozoa were found, and spermatids alone were used for injection. The fertilization rate was 38.1% per injected oocyte, and one pregnancy resulted. In 20 cycles (18.9%), the oocytes were not injected due to failure to find either spermatozoa or spermatids. The relationship between the previous histopathological reports and the findings on the day of oocyte retrieval was investigated (Table VI). Spermatozoa were retrieved in 22 out of 28 (78.6%) cases previously diagnosed as partial spermatogenic arrest, in 19 out of 25 (76%) mixed SCO, in 7 out of 21 (33%) complete arrest at early spermatids, in 4 out of 17 (23.5%) classical SCO, and in 2 out of 9 (22%) cases of complete arrest at primary spermatocytes. Spermatozoa were also retrieved from one case with hyalinization and five cases of hypospermatogenesis.

The fertilization and pregnancy rates were significantly higher in the obstructive group when compared to the non- obstructive group ($P < 0.05$; Table III).

In total, 53 clinical pregnancies resulted from 241 cycles (22%): 35 singles, nine sets of twins, three sets of triplets, and one set of quadruplets; the other five patients miscarried in the first trimester (9.4%). A total of 43 children were born, comprising two sets of triplets,

eight sets of twins and 21 singles. The spermatid pregnancy resulted in the delivery of a healthy boy with normal karyotyping.

DISCUSSION

At the beginning of our ICSI programme for obstructive azoospermic patients, spermatozoa were retrieved using the MESA technique, as described by Silber et al. (1994) and Tournaye et al. (1994). When the epididymis was absent or fibrosed, testicular biopsy was performed to retrieve spermatozoa for ICSI. The fertilization and clinical pregnancy rates at our centre using epididymal spermatozoa were comparable to the results of ICSI combined with TESE (Mansour et al., 1996a,b). Due to the simplicity of the TESE technique we shifted to its routine use in cases of obstructive azoospermia, except in the presence of spermatocele or when the epididymis was found to be distended, where it is easier to obtain spermatozoa through PESA (as described by Craft et al., 1995; Collins et al., 1996; Hirsh et al., 1996; Tsirigotis et al., 1996). Encouraged by the initial report on the use of ICSI in non-obstructive azoospermia (Devroey et al., 1995), ICSI and TESE have also been extended to non-obstructive azoospermia in our programme.

The results of this study demonstrate that ICSI, in combination with surgically retrieved spermatozoa, achieves good fertilization and pregnancy rates (56 and 30.4%) in obstructive azoospermia, comparable to other successful programmes (Silber et al., 1994; Tournaye et al., 1994).

Our data show that testicular spermatozoa from cases with obstructive azoospermia used for ICSI achieve good fertilization and clinical pregnancy rate (54.4 and 31.9%) which are comparable to those with epididymal spermatozoa (59.5 and 27.3%). These findings are in agreement with a previous study by Silber et al. (1995) that concluded that epididymal and

testicular spermatozoa yield similar fertilization, cleavage and ongoing pregnancy rates with ICSI in cases of obstructive azoospermia. Also, these results are comparable to the fertilization rate and pregnancy rate (61 and 30.5%) obtained with ICSI using ejaculated spermatozoa in our centre (Mansour et al., 1996a,b). It has also been shown by Gil-Salom et al. (1995) that high fertilization, cleavage and pregnancy rates can be achieved with intracytoplasmic testicular sperm injection, reaching values comparable to those of ICSI using ejaculated spermatozoa.

In this study, we compared the results of ICSI in acquired obstructive azoospermia with those of azoospermia due to CAVD. The fertilization rate was not significantly different: 56.6 and 55.2% in the acquired obstruction and CAVD groups respectively. However, the pregnancy rate was significantly higher ($P < 0.05$) in the acquired obstruction group (37%) than in the congenital group (20.4%). In a previous study by Chen et al. (1995) that compared acquired and congenital obstructive azoospermia, there was also no significant difference in the fertilization rate. However, contrary to our findings, they concluded that the congenital group seemed to have a stronger tendency to achieve pregnancy than the acquired obstruction group. It should be mentioned, however, that their study was of a small series.

It was reported recently that the fertilization rate and cleavage rates in patients with severe spermatogenic defects were similar to those observed using testicular spermatozoa from men with normal spermatogenesis in obstructive azoospermia (Devroey et al., 1995; Tournaye et al., 1995). In a recent study, Devroey et al. (1996) emphasized that even in cases of the most severe testicular failure, such as Sertoli cell-only syndrome, patients now must be counseled that if a few spermatozoa are found, the chance of pregnancy for the couple is no different than that for a couple with normal spermatogenesis. Indeed, the results of our study demonstrated that the fertilizing ability of testicular spermatozoa obtained from obstructive azoospermic patients was significantly higher than those obtained from non-obstructive

cases. Even in the 60 cycles (out of the 106 cycles of non-obstructive azoospermia) in which enough spermatozoa were successfully retrieved to inject all the metaphase II oocytes, the fertilization and pregnancy rates were significantly lower than in the obstructive group. These results confirm a recent study by Kahraman et al. (1996a,b) that demonstrated a significantly higher fertilization rate in the obstructive group as compared to the non-obstructive group. This may be due to the fact that, in non-obstructive azoospermia, there is severe impairment of spermatogenesis, and even testicular failure. One hypothesis is that it might have a genetic origin (Smith et al., 1979; Vogt et al., 1992; Martin-du-Pan and Casnpana, 1993; Chandley, 1995; Vogt et al., 1995). Non-obstructive azoospermic patients, and those with severe idiopathic oligozoospermia, may be suffering from a genetic defect or a genetically determined barrier to reproduction (Vogt, 1995). Therefore, it is not surprising that, despite succeeding in extracting live spermatozoa in non- obstructive cases of azoospermia, the fertilization and pregnancy rates are significantly lower than those of obstructive azoospermia.

Our study demonstrated that in 67% of the cycles involving non-obstructive azoospermia spermatozoa were found and used for ICSI, achieving a fertilization rate of 39%. In 15 (14.2%) cycles, no spermatozoa were found, and spermatids were used for the injection, with a resulting fertilization rate of 38%. In 20 out of 106 cases (19%), neither spermatozoa nor spermatids were found after an extensive search and the oocytes were not injected. Of interest to this study is the relationship between previous histopathological reports and the rate of sperm retrieval (Table IV), which was 78.6% in cases of partial spermatogenic arrest, 76% in mixed SCO, 33% in cases of complete arrest at early spermatids, 23.5% in classical SCO, and in 22% in complete arrest at primary spermatocytes. In a recent study by Tournaye et al. (1997), spermatozoa were successfully recovered in some patients with tubular sclerosis (seven out of 18), SCO (55 out of 112) and maturation arrest (39 out of 76). In another study

by Kahraman et al. (1996a,b), ICSI combined with TESE was done for 29 men with non-obstructive azoospermia, and enough spermatozoa could be retrieved for ICSI in 14 patients. It is clear from these data that even in non-obstructive azoospermia there are usually tiny foci of spermatogenesis that allow TESE with ICSI to be performed in cases that were previously considered absolutely hopeless. Therefore, testicular biopsy should not be avoided in an azoospermic patient only on the basis of elevated serum FSH concentrations, at least not until other markers more specific than FSH for the presence of mature spermatozoa within the testes are developed (Carreau, 1995; Martin-du-Pan and Bischof, 1995). Mulhall et al. (1997) demonstrated that 70% of 21 patients with testicular dysfunction and azoospermia had spermatozoa in testicular tissue analysis, and neither patient age nor FSH concentration was predictive of the ability to find spermatozoa. Previous studies on spermatogenesis based on histopathological diagnosis documented that some patients with absolute azoospermia may show some foci of spermatogenesis (Steinberger and Tjioe, 1968; Girgis et al., 1969; Clermont, 1972). Therefore, an extremely low rate of spermatogenesis in the testes will result in absolute azoospermia in the ejaculate, even though there are some spermatozoa being produced. A certain tiny threshold of sperm production is necessary before any spermatozoa appear in the ejaculate (Silber et al., 1996).

In conclusion, ICSI with surgically retrieved spermatozoa achieved fertilization rates and pregnancy rates per cycle respectively of 56 and 30.4% in obstructive azoospermia and 39 and 11.3% in non-obstructive azoospermia. In obstructive azoospermia, the fertilization and pregnancy rates resulting from the use of testicular spermatozoa were similar to those with epididymal spermatozoa, and the pregnancy rate in the acquired obstruction group was significantly higher than in the CAVD group. In 67% of cycles involving non-obstructive azoospermia it was possible to find spermatozoa for ICSI. The fertilization and pregnancy

rates resulting from the use of testicular spermatozoa in obstructive cases were significantly higher than in non-obstructive cases.

Table I. Aetiology of infertility in 241 intracytoplasmic sperm injection cycles for patients with azoospermia

Aetiology	No. of cycles
Obstructive	**135**
Congenital absence of vas deferens	54
Bilateral	43
Unilateral	11
Acquired obstruction	81
Non-obstructive	**106**
Hypospermatogenesis	5
Partial spermatogenic arrest	28
Complete spermatogenic arrest (spermatid)	21
Complete spermatogenic arrest (primary spermatocyte)	9
Sertoli cell-only syndrome (mixed)	25
Sertoli cell-only syndrome (classical)	17
Hyalinization	1
Total	**241**

Table II. Characteristics of patients with obstructive or non-obstructive azoospermia and of their partners. There were no statistically significant differences between the two groups

	Obstructive	Non-obstructive
Number of patients	119	103
Age of the male (years)		
Range	23–55	25–60
Mean ± SD	40.4 ± 6.3	39.6 ± 7.2
Age of the female (years)		
Range	19–42	23–44
Mean ± SD	32.9 ± 4.7	32.5 ± 5.2
Duration of infertility (years)		
Range	2–25	2–27
Mean ± SD	10.5 ± 4.8	9.2 ± 5.6

Table III. Outcome of intracytoplasmic sperm injection (ICSI) for patients with obstructive and non-obstructive azoospermia

	Obstructive			Non-obstructive testicular	Total
	Epididymal[a]	Testicular[a]	Total		
No. of ICSI cycles	44	91	135	106	241
No. of injected oocytes (MII)	471	955	1426	754	2180
No. of fertilized oocytes (2PN)	280	519	799	294	1093
Fertilization rate (%)	59.5	54.4	56[b]	39[b]	50.1
No. of ICSI cycles reaching embryo transfer stage (%)	44 (100)	91 (100)	135 (100)	79 (74.5)	213 (88.4)
No. of clinical pregnancies	12	29	41	12	53
Clinical pregnancy rate					
per cycle (%)	27.3	31.9	30.4[b]	11.3[b]	22
per embryo transfer (%)	27.3	31.9	30.4	15.2	24.9

[a]There were no significant differences between the testicular and epididymal groups of the obstructive azoospermia cycles.
[b]Values with the same superscript were significantly different ($P < 0.05$).
MII = metaphase II; PN = pronuclear.

Table IV. Results of intracytoplasmic sperm injection (ICSI) in acquired obstructive azoospermia, congenital absence of the vas deferens (CAVD) and non-obstructive azoospermia

	Obstructive		
	Acquired	CAVD	Non-obstructive[a]
No. of ICSI cycles	81	54	60
No. of injected oocytes (MII)	839	587	549
No. of fertilized oocytes (2PN)	475	324	218
Fertilization rate (%)	56.6[c]	55.2[c]	39.7[b]
No. of ICSI cycles reaching	81	54	55
embryo transfer stage (%)	(100)	(100)	(91.7)
No. of clinical pregnancies	30	11	11
Clinical pregnancy rate			
per cycle (%)	37[c]	20.4[d]	20.0[d]
per transfer (%)	37	20.4[d]	21.8[d]

[a]Only cases in which enough spermatozoa were found for the injection were included, i.e. 60 out of 106 total.
[b,c,d,e]Values within rows with different superscripts were significantly different ($P < 0.05$).

Table V. Type of germ cell injected and outcome in non-obstructive azoospermia

Type of cell injected	No. of cycles (%)	Fertilization rate (%)	Pregnancy rate per cycle (%)
Spermatozoa	60 (56.6)	39.7	18.3
Spermatozoa + spermatids	11 (10.4)	39	—
Spermatids	15 (14.2)	38.1	6.7
None	20 (18.9)	—	—
Total	106	39	11.3

Table VI. Relationship between the histopathological testicular biopsy report and the type of injected germ cell in the non-obstructive azoospermia group

		Spermatogenic cell used on the day of oocyte retrieval			
Histopathology of previous biopsy report	No. of cycles	Spermatozoa	Spermatozoa + spermatids	Spermatids	No
Hyalinization	1	1	—	—	—
Sertoli cell-only (classical)	17	4	—	2	11
Sertoli cell-only (mixed)	25	19	2	1	3
Complete arrest at primary spermatocytes	9	2	—	2	5
Complete arrest at early spermatids	21	7	6	7	1
Partial spermatogenic arrest	28	22	3	3	—
Hypospermatogenesis	5	5	—	—	—
Total	106	60	11	15	20

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Chapter 10

Multifetal pregnancy reduction: Modification of the technique and analysis of the outcome

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Abstract

Objective: To modify the technique of multifetal pregnancy reduction and to study the outcome of reduced twins in comparison with nonreduced twins and high-order multiple gestations.

Design: Prospective controlled study.

Setting: The Egyptian IVF-ET Center, Cairo.

Patient(s): Seventy-five patients with high-order multiple pregnancies resulting from assisted reproduction. Controls were 40 nonreduced twin pregnancies and 22 high-order multiple gestations.

Intervention(s): Transvaginal ultrasonically guided multifetal pregnancy reduction was performed. The first 30 cases were done using KCl as a cardiotoxic agent. The modified technique was used for the last 45 cases at an earlier gestational age (approximately 7 weeks) by eliminating the use of KCl and by aspirating the embryonic parts.

Main Outcome Measure(s): Miscarriage rate, gestational age at delivery, birth weight, and pregnancy complications.

Result(s): Using the modified technique, the miscarriage rate was 8.8% and 41 patients delivered between 32 and 39 weeks of gestation (mean ± SD,36.9 ± 2.45 weeks). The mean (± SD) birth weight was 2,450.51 ± 235.44 g. The miscarriage rate, fetal wastage rate, mean gestational age, and mean birth weight were similar in reduced and nonreduced twins and were significantly better than in nonreduced triplets and quadruplets.

Conclusion(s): The modified technique of multifetal pregnancy reduction significantly improved outcomes, which were similar to those of nonreduced twins resulting from assisted reproduction and significantly better than those of nonreduced triplets and quadruplets. (Fertil Steril® 1999;71:380-4. ©1999 by American Society for Reproductive Medicine.)

INTRODUCTION

During the past 20 years, the use of ovulation-induction drugs for various assisted reproduction techniques has increased significantly. This practice has led to a marked increase in the multiple pregnancy rate, with all of its complications. To reduce the incidence of multiple gestations, most IVF centers reduce the dose of ovulation induction drugs and limit the number of embryos per transfer. However, multiple pregnancies are still unavoidable.

Multifetal pregnancy reduction was introduced to avoid the increased incidence of abortion and premature labor associated with multiple pregnancies (1). Since the beginning of our IVF program in 1986, multifetal pregnancies have been a problem. Multiple gestation is a high-risk condition because of the marked increase in maternal complications and perinatal mortality and morbidity. Faced with potentially tragic situations such as the agony of infertile couples who lose their premature infants, we introduced multifetal pregnancy reduction in our center in 1990 after obtaining institutional review board approval.

We report our experience in performing 75 cases of multifetal pregnancy reduction for high-order multifetal pregnancies with special emphasis on our technique, which is a modification of previously described techniques. We also analyzed the obstetric outcomes to see whether this technical improvement has made this method a better approach for high-order multifetal pregnancies. A control group consisted of 40 nonreduced twin pregnancies resulting from assisted reproduction. Pregnancy outcomes were also compared with a group of 22 patients with high-order multiple gestations that did not undergo reduction.

MATERIALS AND METHODS

From January 1990 to December 1997, we counseled 94 patients with high-order multiple gestations for embryo reduction after obtaining institutional review board approval. Seventy-five women underwent the procedure. Sixty-eight patients were from our center and 7 patients were referred to us from other doctors. Forty-five gestations were triplets, 27 were quadruplets, and three were quintuplets. All the patients were counseled to reduce the number of sacs to two. Two patients requested the reduction to a single sac, but we convinced them to leave two viable fetuses.

Embryo reduction was done as an outpatient procedure under IV general anesthesia using propofol 10% (Diprivan; Zeneca Limited, Macclesfield, Cheshire, United Kingdom). The vagina was prepared with 10% povidone iodine and was then thoroughly rinsed with sterile saline solution and drying to remove any traces of povidone iodine. Transvaginal ultrasound was used (Bruel and Kaejer model 3536 with transvaginal transducer model 8538; Naerum, Denmark). The number of gestational sacs with pulsating echoes was confirmed.

The most accessible gestational sac was chosen, and the fetal heart was visualized and aligned with the puncture guideline on the screen. An oocyte retrieval needle (Labotect cat. no.322109; Gottingen, Germany), was advanced sharply toward the fetal heart. For successful puncture of the fetal heart, the ultrasound transducer should be introduced deeply inside the vagina so as to firmly stretch the vaginal vault before introducing the needle. It is sometimes helpful to support the uterus lightly in the suprapubic region.

In the first 30 patients in this series, we injected 0.5-3 mL of KCl (15%) according to a previously described technique (2). We modified this technique by eliminating the use of KCl completely. After introduction of the needle into the fetal echoes, we applied suction (using a 20-mL syringe) repeatedly until all or most of the embryonic parts were aspirated. We did not

aspirate any amniotic fluid. In most cases, the fetal echoes disappeared completely and the sac remained of the same size or slightly smaller, but empty of fetal parts. After making sure that the fetus was aspirated, or, if not completely aspirated, that there were no more pulsations, we withdrew the needle.

The procedure was repeated for another gestational sac (or two) in the case of quadruplet or quintuplet pregnancies. The patients were given 1 g of IV cefotaxime (Claforan; Hoechst Orient SAE, Cairo, Egypt) before the procedure and were discharged after 3 hours. Follow-up ultrasound examination was done 1 week after the procedure. All patients received routine antenatal care and follow-up of the pregnancy except for those who traveled abroad. Outcome data was obtained for all pregnancies.

An age-matched group of 40 patients with nonreduced twin pregnancies resulting from assisted reproductive techniques was chosen as a control. In addition, we compared outcomes between the study group and 22 high-order multiple pregnancies that did not undergo fetal reduction.

Student's t-test was used to compare the mean gestational ages and birth weights. Fisher's exact test was used to compare the miscarriage rates.

RESULTS

The gestational age at reduction ranged between 6 and 9 weeks (mean ± SD of 7.2 ± 0.1 weeks). At first the procedure lasted 5-10 minutes per gestational sac, but after the clinician gained more experience, the procedure took only 1-5 minutes per sac. In addition, in the beginning 0.5 to 3 mL of KCl was injected (n = 30 cases), but the procedure was later done without use of KCl (n = 45). No immediate complications of the procedure (e.g., vaginal bleeding) occurred, and all the cases were technically successful. No chorioamnionitis or septic abortion occurred.

We analyzed the 45 cases using the modified technique. First-trimester miscarriage occurred in one patient and second-trimester miscarriage occurred in three patients (miscarriage rate of 8.8%). Forty-one patients delivered 73 infants (nine deliveries were singletons). The mean (± SD) gestational age was 36.9 ± 2.45 weeks, and the mean (± SD) birth weight was 2,450.51 ± 235.44 g. One stillbirth and one neonatal death occurred.

The first group of patients (n = 30) that had the procedure with KCl had nine miscarriages (30%), three stillbirths, and two neonatal deaths. Twenty-one patients delivered 41 infants (3 singletons, 2 triplets, and 16 twins). The mean (± SD) gestational age was 36.3 ± 1.64 weeks, and the mean (± SD) birth weight was 2,198.06 ± 656.3 g.

The outcome of an age-matched group of nonreduced twin pregnancies resulting from medically assisted reproduction was compared with our study group in which we did not use KCl (Table 1). The study and control groups were similar in maternal age (32.6 versus 33.4 years, respectively) and duration of infertility (5.4 versus 6.1 years, respectively). There were no statistically significant differences in the abortion rate, mean gestational age, mean birth weight, and fetal wastage between the groups.

The outcomes of high-order multiple pregnancies that did not undergo reduction were compared with the outcomes after multifetal reduction with the modified technique (Table 2). There was a significantly higher abortion rate and fetal wastage rate and a significantly lower gestational age and birth weight when no fetal reduction was performed.

DISCUSSION

In this study, we modified the technique of multifetal pregnancy reduction by avoiding completely the use of any cardiotoxic substance such as KCl. Our approach depends mainly on performing the procedure early (as soon as fetal pulsations are confirmed), usually between 6 and 8 weeks of gestation. At this stage, it is not difficult to aspirate all or most of the embryonic parts, leaving the gestational sac almost intact and full of amniotic fluid but empty of any embryonic tissues.

Itskovitz et al. (3) described two cases of multifetal reduction without the use of an embryotoxic substance. Their technique differed from ours in two respects. First, they applied suction only to stop fetal pulsations and not to aspirate the embryonic tissues. Second, they partially aspirated the amniotic fluid.

The rate of miscarriage in this study using the modified technique was 8.8%. This is less than the 12.6% loss rate reported by Sebire et al. (4) in their series of 127 multifetal pregnancies undergoing embryo reduction and also less than the 13.7% loss rate reported by Evans et al. (2) in a multicenter study of 380 multifetal embryo reductions.

In the first 30 cases when we used KCl as an embryotoxic substance, we had a high fetal wastage rate (30%). One explanation for the lower miscarriage rate using the modified technique is that we performed it after gaining more experience and at an earlier gestational

age. It has been reported that it is feasible to complete the procedure as early as 6 weeks' gestation, although it might be preferable to wait until 8 weeks or later for fear of the natural phenomenon known as "vanishing twin." We recommend performing the procedure as early as possible without excessive concern about the phenomenon of vanishing twins because two gestational sacs will remain. Even if one sac vanishes, a singleton pregnancy will result. In this series of 75 fetal reductions to twins, the pregnancies continued as a singleton in 12 cases (16%)

Another factor that may have played a role in reducing the miscarriage rate using the modified technique is that we aspirated most of the embryonic tissues at an earlier stage. Aspiration leaves a minimal amount of necrotic tissue, which may have detrimental effects on the remaining gestational sacs. In the beginning, it was difficult to imagine that it was possible to aspirate an embryo of 6-8 weeks with visible pulsations. However, the embryo at this stage is composed of three primary germ layers (ectoderm, endoderm, and mesoderm) folding to form the head, tail, and lateral body folds, and these soft tissues and membranes are easily removed with repeated suction.

One of the causes of pregnancy loss in reduced twins is the development of an inflammatory response to the resorbing necrotic fetoplacental tissues, with the resulting release of cytokines and prostaglandins (4). High concentrations of α-fetoprotein (AFP) are found in the amniotic fluid of twin pregnancies after spontaneous death of one of the fetuses, as reported by Bass et al. (5) and in reduced twins.

Abbas et al. (6) reported that after reduction of multifetal pregnancies, the maternal serum AFP concentration increases in proportion to the amount of dead fetoplacental tissue, and this increase persists for several months after the procedure. We believe that performing multifetal reduction at an earlier stage (i.e., before 8 weeks of gestation) probably decreases the concentration of AFP remaining after the reduction. This has yet to be confirmed.

Another reason for the lower miscarriage rate is that the injection of KCl is not strictly limited to the fetal heart. During injection, the fetus is sometimes pushed away from the needle and KCl diffuses into the amniotic sac; consequently it may diffuse to the adjacent gestational sacs. Toxic effects of KCl on the remaining fetuses have been reported by Tabsh et al. (7) and Wapner et al. (8).

In comparison with the nonreduced twins, the outcome of the twins resulting from the modified reduction technique was not significantly different with regard to the miscarriage rate, fetal wastage rate, mean gestational age, and birth weight. Nonreduced twins that were chosen as a control in this study resulted from IVF or intracytoplasmic sperm injection. It has been shown that twin pregnancies conceived by assisted reproductive techniques resulted in more frequently discordant birth weight and low birth weight compared with those conceived spontaneously. In 1996, Smith-Levitin et al. (9) demonstrated that reduced twins were similar to nonreduced twins conceived with assisted reproduction in all variables studied.

It was also noted in this series that the early (<12 weeks) miscarriage rate was only 2.6%, which is similar to that of early amniocentesis in singleton pregnancies (2.2%). This leads to the assumption that most miscarriages associated with multifetal reduction are not the consequence of spontaneous loss, nor are they directly due to the procedure and the use of needles in the reduction.

The mean gestational age at delivery (36.9 ± 2.45 weeks) of the twins resulting from the modified technique was similar to that of the nonreduced twins conceived by assisted reproduction (36.5 ± 2.58 weeks). This finding is probably due to the minimal amount of necrotic tissues remaining after reduction and thus the smaller likelihood that their resorption could trigger labor.

Compared with the outcome of high-order multiple pregnancies, our results indicate that the gestational age at birth and mean birth weight increased after multifetal reduction. The miscarriage rate and perinatal mortality also decreased significantly with fetal reduction. Among six cases of quadruplets that were managed without embryo reduction, three (50%) resulted in late miscarriage. At delivery, the mean (± SD) birth weight was 1,400 ± 316 g and the mean (± SD) gestational age was 32.67 ± 2.31 weeks. The fetal wastage rate (miscarriage, stillbirth, and neonatal death) was 66.6%.

The control group also included 16 cases of nonreduced triplets. Miscarriage occurred in four cases (25%). At delivery, the mean (± SD) birth weight was 2,042.8 ± 500.5 g and the mean (± SD) gestational age was 34.46 ± 2.787 weeks, which were significantly lower than those of the reduced twins. Five infants were stillborn and five died in the neonatal period (45.8% fetal wastage). This very high fetal wastage rate in triplets and quadruplets used as a control group in this study was mainly due to prematurity and low birth weight.

Bollen et al. (10) and Smith-Levitin et al. (9) demonstrated that multifetal pregnancy reduction gave better outcomes than expectant management of triplets. In a large British study by Botting et al. (11), the perinatal mortality rate was 41.6% in sextuplets, 21.9% in quintuplets, 20% in quadruplets, and 16.4% in triplets.

Multifetal pregnancy reduction raises considerable ethical and religious debates. It is a difficult situation for both the physician and the patient. Our study was approved by our institution' s ethics committee, but it was difficult to counsel patients because of the lack of sufficient data about the procedure. We did not know initially what the chances would be of successfully performing the procedure or the risks of long-term effects of the procedure on the remaining pregnancy. The emphasis was placed on the incidence of early pregnancy loss and the perinatal complications of prematurity. We also emphasized that the procedure could result in total loss of the pregnancy and that the future risks were still unknown.

Our approach to counseling has evolved over the years. At present, we inform patients that the chance of successfully performing the procedure with minimal risk is very high and that the outcome of reduced twins is significantly better than for nonreduced high-order multiple pregnancies. However, the long-term complications and potential risks of the procedure are still uncertain.

TABLE 1

Analysis of the results of reduced and nonreduced twins.

Group	No. of cases	Miscarriage <12 wk	Miscarriage >12 wk	Miscarriage Rate (%)	No. of deliveries	No. of live-born infants	Mean gestational age (wk)	Mean birth weight (g)	No. of stillborn infants	No. of neonatal deaths
Reduced twins	26 triplets 17 quadruplets 2 quintuplets 45 total	1	3	8.8*	41	72 (9 singletons)	36.9 ± 2.45†	2,450.51 ± 235.44‡	1	1
Nonreduced twins	40	1	3	10*	36	62 (5 singletons)	36.5 ± 2.58†	2,316.04 ± 603.09‡	1	1

Note: Data are expressed as *n*, %, or mean ± SD.
* $P = .211$ (not significant).
† $t = 0$ (not significant).
‡ $t = 0.75$ (not significant).

TABLE 2

Analysis of the results of reduced twins and nonreduced triplets and quadruplets.

Group	No. of cases	Miscarriage <12 wk	Miscarriage >12 wk	Miscarriage Rate (%)	No. of deliveries	No. of live-born infants	Mean gestational age (wk)	Mean birth weight (g)	No. of stillborn infants	No. of neonatal deaths
Reduced twins	26 triplets 17 quadruplets 2 quintuplets 45 total	1	3	8.8*	41	72 (9 singletons)	36.9 ± 2.45*	2,450.51 ± 235.44*	1	1
Nonreduced triplets	16	—	4	25*	12	29	33.46 ± 2.787*	2,002.8 ± 500.5*	5	5
Nonreduced quadruplets	6	—	3	50*	3	10	32.67 ± 2.31*	1,400 ± 316.2*	2	2

Note: Data are expressed as *n*, %, or mean ± SD.
* $P < .05$.

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Chapter 11

General Discussion

IVF is a delicate and complicated procedure that depends on many factors for success. Taking utmost care of minute details is essential. Our group was interested in conducting research in several areas that are believed to have an impact on the success rate of IVF. The research was focused on:

I *Patient preparation before starting the IVF cycle:*

a- The diagnosis and treatment of hydrosalpinges.

Our group reported for the first time in the literature that the presence of fluid in the uterine cavity in association with hydrosalpinges was a possible hindrance to implantation (1). It was suggested that in such cases, closing the cornual end of the tube combined with a neosalpingostomy could be a possible solution. It was also suggested that transvaginal US guided aspiration or a salpingectomy may improve the IVF outcome. Following this report by our group, several other investigators reported that IVF outcomes were in fact worse in the presence of hydrosalpinges (2-4). Moreover, it was demonstrated that hydrosalpinges adversely affect implantation and pregnancy rates in donor oocyte cycles (5) and cryopreserved embryos (6). In a recent large study that included 1000 patients with tubal factor infertility, the authors concluded that there was a decrease in the implantation rate and an increase in the pre-clinical miscarriages in patients with hydrosalpinges compared to tubal factor patients without sonographic evidence of dilated tubes (7). The exact mechanism for the negative effect of hydrosalpinges is not known. Different theories have been studied including the toxic effect of hydrosalpinx fluid on the embryos as well as on endometrial receptivity (8-11), or simply a mechanical hindrance of the hydrosalpinx fluid to implantation (1,2,12). However, other studies have shown that embryo development was not impaired by hydrosalpinx fluid (13,14). A recent debate on the possible reasons of the negative effect of hydrosalpinx on IVF results discussed all the above mentioned mechanisms and concluded that the hydrosalpinx is of crucial

importance, but the actual mechanism of action needs to be clarified (15). Although the exact mechanism is not known, there is definitely a negative effect of hydrosalpinges on IVF results. Moreover, several retrospective studies have proven the positive effect of salpingectomy on IVF results for patients with hydrosalpinges (3,16,17). On the basis of these retrospective studies IVF programs began counseling patients with hydrosalpinges to undergo a salpingectomy before starting IVF. However, one should not offer a major surgical intervention such as salpingectomy unless it is based on a proper prospective randomized study. The first prospective randomized trial was a pilot study by Déchaud et al, (18) which showed a positive effect of salpingectomy on implantation rates. Recently, Strandell and group did a large multicenter prospective randomized study on 204 patients (19). The results of their study demonstrated that in patients with hydrosalpinges, salpingectomies improved the pregnancy rates. Patients with bilateral hydrosalpinges, especially if ultrasonically visible gained a clear benefit from a salpingectomy. Since the publication of this study, we routinely counsel our patients with hydrosalpinges to undergo a salpingectomy before starting the IVF cycle. It is difficult for young patients to accept having their tubes removed even if they were told that the chances of spontaneous pregnancy are almost nil. It seems preferable in those cases only to do salpingostomy to leave the patient with an open tube. Proximal tubal occlusion and distal fenestration may be another option (1, 20). However, further studies need to be done to investigate the efficacy of distal tubal opening with or without proximal ligation compared to salpingectomy in such cases. Recently, the cumulative results after salpingectomy in the study by Strandell et al., was published (21). The results of the cumulative cycles strengthened the recommendation for a laparoscopic salpingectomy prior to IVF in patients with ultrasound-visible hydrosalpinges (21).

Moreover the same authors conducted another randomized controlled study in which they demonstrated that removal of hydrosalpinx, as a prophylactic laparoscopic procedure does not compromise ovarian function (22).

b- Performing a dummy embryo transfer in an attempt to improve the embryo transfer technique.

We have demonstrated in a randomised study that performing a "dummy" or "trial" embryo transfer before the start of an IVF cycle significantly improves the implantation and pregnancy rates (23). It is a simple procedure that helps in determining the most suitable kind of catheter to be used for ET, and allows us to measure the length and direction of the uterine cavity. It is also helpful in discovering any unanticipated difficulty such as cervical stenosis, anatomical distortion of the cervix from previous surgery, or cervical fibroid. It is like performing a rehearsal before the real transfer. Following this study we have been performing dummy ET routinely for all our patients before starting the IVF cycle and right before the embryo transfer. Many IVF centers routinely perform dummy ET, however, the published data on this is minimal. In one study, it was reported that mock ET was performed immediately before the real transfer on 113 patients and they achieved a 45% pregnancy rate (24).

In another prospective study the actual ET was simulated using a methylene blue dye to study the effect of some factors on the rate of uterine expulsion of the dye (25). It was demonstrated that the dye was extruded at the external cervical os, at least partially, in 42% of all cases. This may indirectly indicate that in the case of the actual

ET, some of the patients might have lost their chance of pregnancy. The results of this study are similar to the results of another study in which the authors used a radio-opaque dye, mimicking the embryo transfer (26). The authors found that the dye remained primarily in the uterine cavity in only 58% of the cases. In the rest of the cases, the dye was extruded from the cervix or into the tubes. Consequently, it is possible that the embryos may be extruded partially or totally after embryo transfer (27, 28). However, if the embryo was extruded into the tube, it may not necessarily interfere with implantation in patients with healthy tubes. In our study, the effect of three factors on the rate of extrusion of the dye was investigated: 1-, the kind of ET catheter used, 2-the presence of cervical mucus, 3- the presence of air bubbles in the catheter. The results of the study demonstrated that the use of soft catheters was associated with a significantly reduced rate of extrusion of the dye compared to the use of more rigid ones. Regarding the effect of cervical mucus, the dye appeared at the external cervical os at a significantly higher rate when the cervical mucus was not removed. The presence of air bubbles in the catheter did not affect the rate of extrusion of the dye.

Several other studies have been done comparing different kinds of catheters and they all demonstrated that soft catheters are the best in terms of pregnancy rates (29-31). The stimulus of the catheter passing through the internal cervical os can initiate contractions probably mediated by the release of prostaglandins (32). The more rigid the catheter is, the more likely that it will agitate the cervix. The presence of cervical mucus can be a serious obstacle in proper embryo replacement. It can plug the tip of the catheter, causing difficulty in delivering the embryos inside the uterine cavity, especially with such a small volume of culture media to inject the embryos. Moreover, the embryos can stick to the mucus around the ET catheter and be dragged

out during withdrawal of the catheter. If the mucus is pushed or injected higher in the uterine cavity, it may interfere with implantation. In a large study analyzing 1204 ET procedures, it was shown that the embryos were much more likely to be retained in the catheter when the ET catheter was contaminated with mucus or blood (33).

II Optimizing fertilization:

In 1992, a breakthrough occurred in the field of IVF through the introduction of intracytoplasmic sperm injection (ICSI) (34). Our IVF program quickly adopted the technique of ICSI in order to improve fertilization. We conducted a number of studies on modifying the technique of ICSI itself (35) as well as studying the effect of different semen parameters (36) and the use of epididymal and testicular spermatozoa in ICSI (37, 38).

We have demonstrated that the fertilization and pregnancy rates in ICSI were not affected by different semen parameters as long as morphologically normal living sperm could be used for the injection (36). Other investigators studied the relationship between the sperm parameters and outcome of ICSI, and found that none of the single sperm parameters, such as concentration, progressive motility, or morphology, correlated with the outcome (39, 40). The technique of ICSI enabled men with very few living spermatozoa in their ejaculates to achieve fertilization and parenthood (41, 42). In our study it was also demonstrated that patients who previously failed fertilization with IVF had successful fertilization with ICSI. ICSI offered these patients an equal chance of fertilization compared to patients with other indications (43).

We have also demonstrated that the use of epididymal and testicular spermatozoa for ICSI in cases of obstructive azoospermia provided an efficient method for achieving fertilization and pregnancy (37). Microsurgical epididymal sperm aspiration (MESA) was the first method described for surgical retrieval of spermatozoa in cases of obstructive azoospermia (44). The

introduction of ICSI has made fertilization very efficient and precise, to the extent that a very low number of spermatozoa are required to inject all the oocytes retrieved for the procedure (45). High fertilization and pregnancy rates have been reported with the combination of MESA and ICSI (45-47). However, the technique is lengthy, requires general anaesthesia, special skills, and equipment. For these reasons, percutaneous sperm aspiration (PESA) was introduced (48). Until recently, the fertilizing capacity of testicular spermatozoa was not explored. In 1993, it was reported that the use of testicular spermatozoa in ICSI for cases of obstructive azoospermia could achieve fertilization and pregnancy (49-51). Following our study (37) many of our patients with obstructive azoospermia were encouraged to undergo ICSI. Such patients were considered absolutely infertile, and no sperm donation is allowed in our country.

Furthermore, our group investigated the technique of performing injection of the sperm inside the oocyte. In a prospective randomized study on sibling oocytes we studied the effects of cytoplasmic aspiration versus non-aspiration on the rate of oocyte damage, fertilization and embryo quality (35). Cytoplasmic aspiration has been considered to be an integral part of the ICSI procedure and an essential step for oocyte activation (42). Moreover, vigorous aspiration of the oocyte cytoplasm has been reported to be pivotal for the success of ICSI (52). However, in our study it was demonstrated that cytoplasmic aspiration before sperm injection was not essential for oocyte activation, as it did not improve the fertilization rate. It was only a way of making sure of piercing the oolemmal membrane. Moreover, it significantly increased the rate of oocyte damage. Also, our technique of no aspiration resulted in a significantly higher percentage of grade I embryos compared to cytoplasmic aspiration. Our report was the first in the literature that demonstrated that cytoplasmic aspiration in the ICSI procedure is not essential for oocyte activation.

In another study by our group (38) the results of ICSI were compared in: 1- obstructive versus non-obstructive azoospermia, 2- testicular versus epididymal spermatozoa in obstructive azoospermia. The results have demonstrated that ICSI using spermatozoa from patients with acquired obstructive azoospermia resulted in significantly higher fertilization and pregnancy rates compared to congenital absent vas deferens (CAVD) and non-obstructive azoospermia. Our data demonstrated that in obstructive azoospermia testicular spermatozoa used for ICSI achieved fertilization and pregnancy rates comparable to those obtained with epididymal spermatozoa, which is in agreement with other studies (51). Also, in this study we compared the results of ICSI in acquired obstructive azoospermia with those of CAVD. The fertilization rate was not significantly different, however the pregnancy rate was significantly higher in the acquired obstructive group. In another study comparing acquired and congenital obstructive azoospermia, there was also no significant difference in the fertilization rate (53). However, contrary to our findings, the authors concluded that CAVD patients seemed to have a stronger tendency to achieve pregnancy than the acquired obstructive group. Recently with a large number of cases reaching 1000 cycles (448 obstructive azoospermia) of ICSI using surgically retrieved spermatozoa, we did not find any significant difference in the fertilization and pregnancy rates between acquired obstructive and BCAV patients (Tables 1, 2). Moreover the results of our study demonstrated that the fertilizing ability of testicular spermatozoa obtained from obstructive azoospermia was significantly higher than those obtained from non-obstructive cases. These results confirm other studies in the literature (54). This may be due to the fact that, in non-obstructive azoospermia there is a severe impairment of spermatogenesis, and even testicular failure, which might have a genetic origin (55). Finally, in this study we have demonstrated that spermatozoa could be retrieved in 67% of the testicular biopsies obtained from non-obstructive azoospermic patients. It is clear from these data that even in non-obstructive

azoospermia there are usually tiny foci of spermatogenesis that allow TESA with ICSI to be performed in cases that were previously considered hopeless. Therefore, we do not deny patients with non-obstructive azoospermia the chance to undergo ICSI.

III Multifetal pregnancy reduction as an attempt to improve the outcome:

The use of ovulation induction drugs for different assisted reproduction techniques has increased considerably during the past 20 years. Consequently, this practice has resulted in a marked increase in the multiple pregnancy rates with all its complications. Most IVF programs have reduced the dose of ovulation induction drugs and limited the number of embryos for transfer in an attempt to reduce the incidence of multiple gestations.

However, the problem of high order multiple pregnancies is still not completely avoidable. Multifetal pregnancy reduction was introduced to avoid the increased incidence of abortion and premature labor associated with multiple pregnancies (56). A modification of the previously described technique of multifetal reduction was introduced by our group (57). The modified approach depends mainly on performing the procedure early, usually between 7 and 8 weeks of gestation and eliminating the use of any cardiotoxic substance such as KCl, which may diffuse to the adjacent sacs and induce detrimental effects (58, 59). At this early stage of gestation it is not difficult to aspirate all or most of the embryonic parts, leaving the gestational sac almost empty of embryonic tissues but full of amniotic fluid. This leaves minimal amounts of necrotic tissues, which also might have a detrimental effect on the remaining embryonic sacs (60-62). Using this technique, the miscarriage and perinatal mortality rates decreased significantly compared to high order multiple pregnancies. The gestational age at birth and the mean birth weight significantly improved after multifetal reduction as compared to non-reduced twins and higher order multiple pregnancy (Table 3). The mean gestational age at delivery of multiples reduced to twins was 36.9 ± 2.45 weeks

and the mean birth weight was 2450.51 ± 235.44 g. which was similar to twins conceived by assisted reproduction (36.5 ± 2.58 weeks and 2316.04 ± 603.09 g.). For the past three years, we have been limiting the number of embryos per transfer to a maximum of three in patients less than 40 years old and undergoing their first IVF-ICSI trial. Unfortunately, we are still facing the problem of triplet pregnancy and we routinely offer these patients the option of multifetal reduction. It was also demonstrated by others that multifetal pregnancy reduction gave better outcomes than expectant management of triplets (63, 64). However, in a large study of 1000 consecutive cases of multi-fetal pregnancy reduction, it was demonstrated that the gestational age of delivery for finishing numbers of one, two and three fetuses are similar to that of non-reduced pregnancies (65). In another review it was demonstrated that multi-fetal pregnancy reduction does not have a significant impact on the probability of live birth or on gestational age at delivery for women with triplets conceived with assisted reproductive technology (66).

An over view on how to optimize IVF results:

Numerous measures have been suggested for optimizing IVF results. Only some have been proven to have an impact on the results and will be discussed. The formation of a good staff team is the key to assure that all the procedures of IVF are conducted properly. Patient preparation before starting the IVF cycle is very important. Special consideration should be given to the diagnosis and treatment of hydrosalpinges, (1, 19, 20) the diagnosis and treatment of lower genital tract infection (67, 68) and proper ultrasonic (US) evaluation of the uterine cavity (69-71). Performing a dummy or trial embryo transfer is also important (23). The choice of the most suitable protocol for ovulation induction is important to obtain an

adequate number of high quality oocytes (72). Strict quality control and quality assurance is vital in any IVF program to produce viable embryos (73). Optimizing fertilization has been successfully achieved through the development of ICSI (34). The technique by which the embryos are transferred into the uterine cavity has a significant impact on the results (74). High order multiple pregnancies must be prevented by limiting the number of transferred embryos (75). However, in cases of high order multiple pregnancies, multifetal reduction might be an option to improve the outcome (57). In a recently conducted survey (76), a questionnaire was sent to the 40 leading ART centers in the USA. The questionnaire included 11 factors for optimizing IVF results and the centers were asked to put them in order of importance. The questionnaire was answered by 38 of the 40 centers. The results of the survey showed that the most important factors in optimizing IVF results are: 1- IVF laboratory including quality control, culture media, lab condition, embryologists, culture and grading of oocytes and embryos. 2- Selection and screening methods for quality embryos. 3- Patient selection. 4- Embryo transfer technique. 5- Improving implantation.

The following are some of the factors that are believed to have an impact on IVF success rates in detail.

Quality control in the IVF laboratory:

IVF, from its name, implies that fertilization is done outside the body, which means that human gametes have to be provided with the exact conditions as in vivo. This requires strict rules and regulations to be observed in order to assure quality control. Several procedures have been proposed, however some are practical and valuable in the discovery of the potential pitfalls that might affect the outcome of IVF. Quality control means routine monitoring of all procedures involved in IVF, while quality assurance means evaluation of the results, identifying problems and finding means for correction. Quality control is essential

for daily monitoring and recording of the temperature and gas levels in the incubators, temperature of all heating stages, water baths, and heating blocks. Environmental control is very important in the IVF laboratory. It is advisable to avoid the use of excessive alcohol to disinfect the facility to avoid extraordinary levels of isopropanol in the laboratory (77). The single and most important measure is to use lots of clean fresh air. The process of fresh clean air going into the laboratory requires two kinds of filtration. A HEPE filter to remove biological material such as molds and bacteria, and an activated carbon filter to absorb organic compounds (78). Moreover, new construction in the vicinity of the laboratory should be avoided. Apart from daily measurements and testing of every procedure related to IVF, it is much more important to have a laboratory director who is continuously following every test to be done, and can interpret and understand from the results what may be a potential risk and how to solve it. Moreover, the laboratory director should individually supervise all the embryologists and lab technicians. The most important factor in quality control and assurance is the quality of the personnel working in the laboratory.

Optimizing the technique of embryo transfer:

There is no doubt that one of the most important factors that contributed to the improvement of IVF results is the increased awareness and expertise in performing the embryo transfer. The importance of the technique by which the embryos are transferred into the uterine cavity is very well illustrated by the fact that there is a significant difference in the pregnancy rates, within the same IVF program, according to the clinician performing the transfer (74, 79). Searching Medline on the Internet revealed that the number of scientific publications on human IVF from 1978 till 2001 is 40500. However, the number of scientific publications on

the technique of embryo transfer is only 54 (79). That discrepancy reflects how little attention has been given to the technique of embryo transfer (79). The embryo transfer is routinely done using the transcervical route, which is basically a blind technique and is associated with multiple potential negative factors. The following are some suggestions for optimizing the embryo transfer technique:

I- Avoid the initiation of uterine contractions

The presence of physiological subendometrial movements has been recognized by different groups (80-87). There are slow subendometrial movements that usually benefit implantation and limit it to the upper uterine cavity, especially if the direction is from cervix to fundus (83). Endometrial wavelike activity was observed in 71% of spontaneous cycles (83) as compared to 91% in stimulated cycles (85). It was found out that fundus to cervix waves never occur after ovulation in spontaneous cycles, nor after ovum pick-up in IVF cycles (87). The later the wave direction switch from fundus to cervix to become cervix to fundus, the higher the likelihood of pregnancy in that cycle (87).

The pathological reflex contractions due to aggressive handling during embryo transfer should be avoided. Following embryo transfer, the embryos might be expelled. About 15% of the transferred embryos have been collected from the external os, the tip of the catheter and vaginal speculum after embryo transfer (27). In a study using radio opaque dye it was demonstrated that the dye remained primarily inside the uterine cavity in only 58% of cases (26). In another study done by our group, using methylene blue dye, it was shown that the dye was visualized at the external os in 42% of the cases (25). Myometrial contractile activity was recorded and an overall uterine contraction frequency of 4.3 per minute was found (88). The pregnancy and implantation rates decreased as the frequency of uterine contractions

increased (88). Several precautions should be taken to avoid the initiation of pathological reflex uterine contractions during embryo transfer:

a) *The use of soft catheters:*

Several studies have been done comparing different kinds of catheters for ET and most of them have demonstrated that soft catheters are the best in terms of pregnancy rates (29-31). An ultrasonographic monitoring revealed disruption in the endometrium in 50% of women in whom a Tomcat catheter was used compared to 12.5% with the use of a Wallace catheter (89). Changing from Tomcat to Wallace catheters has been associated with a better pregnancy rate (89). To benefit from the advantages of the softness of the catheter, the outer rigid sheath should minimally be used to stop short of the internal os. The stimulus of the catheter passing through the internal os can also initiate contractions probably mediated by the release of prostaglandins (32).

b) *Avoid touching the uterine fundus:*

Depositing the embryos in the mid fundal area was found to improve the pregnancy rate (90). In another study it was found that transferring the embryos at 6 cm without tracing the position of the fundus improved the pregnancy rates (91). Other groups routinely place the catheter 0.5 cm below the fundus (92). Therefore, individual measurements of the cervical canal and uterine cavity are important to know exactly how much you can introduce the catheter without touching the fundus.

c) *Gentle manipulation:*

As a general rule, ET should be a simple and painless procedure. It has been observed that the use of tissue forceps to hold the cervix triggers uterine contractions (93). The use of soft catheters should be the rule except when it cannot be introduced. Technically difficult transfers were found to be associated with reduced pregnancy

rates (24, 94). This is probably due to stimulation of uterine contractions that might expel the embryos (88).

II. Getting rid of cervical mucus

It has been demonstrated that removing the cervical mucus before a methylene blue dummy embryo transfer significantly reduced the rate of extrusion of the dye at the external cervical os (25). It was also shown that the embryos were much more likely to be retained in the ET catheter if it was contaminated with mucus or blood (33, 94). Therefore it advisable to gentle aspirate the cervical mucous before ET to avoid its introduction with the catheter inside the uterine cavity and the possibility of blocking the tip of the catheter.

III. Proper delivery of the embryos inside the uterine cavity

The most important point in ET is to be absolutely sure that the catheter has passed the internal os and entered the uterine cavity. Soft catheters can sometimes be misleading when they curve inside the cervical canal. With experience, a simple test can confirm that the catheter is in the cavity. Rotating the catheter 360^{o} will show when it is bent if it recoils (79). One important cause of the failure of the catheter to pass the internal os is the lack of alignment between the catheter (straight) and the cervico-uterine angle (curved and sometimes acutely angulated) (79). A simple procedure of gently curving the outer sheath of the catheter will overcome this problem in most of the situations. Performing a dummy ET right before the actual one and revising the previously taken US picture of the uterus will help to find the right direction.

The use of US guidance to facilitate embryo transfer has been described by some IVF programs (95, 96). It has proven to be useful in women with a previously difficult embryo transfer. It has also been found to be simple, reassuring, and it significantly improved the

pregnancy rate (31, 97, 98). However, other investigators found no significant difference in the pregnancy rate between US guidance and clinical touch ET (99, 100). It seems that, using either one of the two techniques; the IVF outcome depends on the experience of the clinician performing the ET.

Avoiding high order multiple pregnancies:

To avoid triplet pregnancy, some European ART centers adopted the policy of transferring only two embryos (101). However, the number of twin pregnancies after transferring only two embryos is still high (102). In a recent study elective single embryo transfer (eSET) was performed for 127 patients achieving a clinical pregnancy rate of 38.6% in fresh embryos and 17.4% in cryo-thawed embryos (75). The cumulative delivery rate per oocyte retrieval was 52.8% and the monozygotic twin rate was 7.6% (75). Results from several centers were presented during an ESHRE campus course (103) and it was recommended that sound clinical trials are needed to clarify several points such as: a) the clinical profile of patients in whom eSET should be considered. b) will the overall pregnancy rate decrease? c) will the financial gain of prevented twins be balanced by the likely needed to perform extra IVF/ICSI cycles?

The problem of high order multiple pregnancies is better solved by prevention rather than multifetal pregnancy reduction. The ideal situation is to transfer one or two embryos only without jeopardizing the pregnancy rate.

Table 1. Results of ICSI using surgically retrieved spermatozoa in congenital and acquired obstructive azoospermia

	Total	Congenital Absent Vas	Acquired Obstruction
Number of cycles	448	175	273
Fertilization rate (%)	54.4	54.7	54.2
Pregnancy rate (%)	34.4	35.4	33.7

Table 2. Results of ICSI using surgically retrieved testicular and epididymal spermatozoa in obstructive azoospermia

	Total	Testicular Spermatozoa	Epididymal Spermatozoa
Number of cycles	448	285	163
Fertilization rate (%)	54.4	53.6	55.9
Pregnancy rate (%)	34.4	33	36.8

Table 3. Analysis of the results of reduced and non-reduced twins, triplets, and quadruplets.

	No. of Cases	Miscarriage <12weeks	Miscarriage >12weeks	Miscarriage Rate%	No. of Deliveries	No. of live born babies	Mean gestational age ± SD (wk)	Mean birth weight ± SD (g)	No. of still born babies	No. of Neonatal deaths
Reduced Twins	26 triplets 17 quads 2 quads 45 total	1	3	8.8*	41	72 (9 singletons)	36.9 ± 2.45*	2,450.51* ± 235.44	1	1
Non-reduced Twins	40	1	3	10	36	62 (5 singletons)	36.5 ± 2.58	2,316.04 ± 603.09	1	1
Non-reduced Triplets	16	--	4	25*	12	29	33.46 ± 2.787*	2,002.8* ± 500.5	5	5
Non-reduced quadruplets	6	--	3	50*	3	10	32.67 ± 2.31*	1,400* ± 316.2	2	2

Data are expressed as n, % or mean ± SD * P< .05

Future Perspectives

Salpingectomy has been shown to improve IVF outcome in patients with hydrosalpinges. Further research is needed to investigate the efficacy of distal opening of the tube with or without proximal ligation as compared to salpingectomy in patients with hydrosalpinges before starting IVF.

For improving the technique of embryo transfer, and decreasing the rate of extrusion of the embryos after embryo transfer, future studies need to be done on experimenting with different uterine relaxant drugs that may decrease the initiation of uterine contractility.

ICSI has significantly improved fertilization and even made it possible in azoospermic patients to achieve parenthood. However, there still are about 35% of non-obstructive azoospermic patients in whom no spermatozoa could be retrieved. Future research should concentrate on the use of diploid spermatogenic cells. In cases of spermatogenic arrest, many primary spermatocytes are present that could be matured in-vitro or be used as diploid cells for injection. After triploid fertilization, one male pronucleus can be removed.

The problem of high order multiple pregnancies should be considered more seriously. Single embryo transfer needs to be further studied to delineate the criteria of patients suitable for single embryo transfer.

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Chapter 12

Summary

Summary

In Chapter 1, the history of IVF was reviewed and an outline about the results of IVF in different parts of the world was written. Different factors that resulted in improving IVF results were discussed with special emphasis on ovarian stimulation protocols, development of US in ovum pick-up techniques, improvement in fertilization due to ICSI, and the development in tissue culture media.

The aim of the work, as illustrated in chapter 2, is optimizing IVF results through the following: First, in patient preparation before starting an IVF cycle, focusing on the diagnosis and management of hydrosalpinges, performing dummy embryo transfers and studying different factors that may affect the embryo transfer. Second, experimenting with the technique of ICSI in an attempt to improve fertilization. The research was focused on modifying the technique of ICSI, studying the effect of different sperm parameters, and using testicular and epididymal spermatozoa in cases of azoospermia. Third, modification of the technique of multifetal pregnancy reduction in cases of high order multiple pregnancies in an attempt to improve the outcome.

In chapter 3, the presence of fluid in the uterine cavity in association with hydrosalpinges was reported as a hindrance to implantation and poor IVF outcome. This was the first report in the literature that was followed by several studies by other investigators and finally led to the conclusion that salpingectomy is recommended in patients with hydrosalpinges before performing IVF.

In chapter 4, we have demonstrated that performing a dummy embryo transfer significantly improved the IVF outcome. It helps in choosing the most suitable kind of catheter, evaluating the length and direction of the uterine cavity, and discovering any unanticipated difficulty.

In chapter 5, the research on the technique of embryo transfer was continued using methylene blue dye. The effect of different factors on the rate of extrusion of the dye was

studied. It was demonstrated that the rate of extrusion of the dye was significantly less when soft catheters were used as compared to more rigid ones and when the cervical mucous was aspirated.

In chapter 6, the effect of different sperm parameters on the outcome of ICSI was studied. It was demonstrated that the fertilization and pregnancy rates in ICSI were not affected by different sperm parameters as long as morphologically normal living sperm was used for the injection. It was also demonstrated that patients who previously failed fertilization with IVF had successful fertilization with ICSI.

In Chapter 7, a study was done on the use of epididymal and testicular spermatozoa in ICSI. It was demonstrated that ICSI using epididymal and testicular spermatozoa in cases of obstructive azoospermia is an efficient method in achieving fertilization and pregnancy.

In Chapter 8, a prospective randomized study was done on sibling oocytes to investigate performing ICSI without cytoplasmic aspiration. The study demonstrated that cytoplasmic aspiration before sperm injection was not essential for oocyte activation because it did not improve the fertilization rate. Moreover, aspiration significantly increased the rate of oocyte damage. The technique of no aspiration resulted in a significantly higher rate of good quality embryos as compared to performing cytoplasmic aspiration before injecting the spermatozoa.

In Chapter 9, a study of the outcome of ICSI was done in obstructive and non-obstructive azoospermia. It was demonstrated that ICSI using spermatozoa from patients with acquired obstructive azoospermia resulted in significantly higher fertilization and pregnancy rates as compared to congenitally absent vas deferens and non-obstructive azoospermia. There was no difference in the fertilization and pregnancy rates using epididymal or testicular spermatozoa in obstructive azoospermia. The results also demonstrated that the fertilizing ability of testicular spermatozoa obtained from non-obstructive azoospermia was significantly lower than those obtained from obstructive azoospermic cases. Finally it was demonstrated that spermatozoa

could be retrieved in 67% of the testicular biopsies obtained from non- obstructive azoospermic patients.

In Chapter 10, a modified technique was described for multifetal pregnancy reduction in cases of high order multiple pregnancy. The modified technique eliminated completely the use of any cardiotoxic substance such as KCl. It is done as early as 7-8 weeks of gestation by transvaginal US guided aspiration of the fetal echoes. Using the modified technique the outcome of the reduced twins was comparable to non-reduced twins.

In Chapter 11, an attempt was done to correlate these chapters with the literature and an overview was given about how to optimize IVF results. It was stressed that IVF is a delicate and complicated procedure that depends on many factors for success. The formation of a good team is the key to assure that every procedure of the IVF is conducted properly. Patient preparation before starting the IVF cycle is very important. Special attention should be given to the diagnosis and treatment of hydrosalpinges, and performing a dummy embryo transfer to choose the most suitable kind of catheter. One should use the most suitable protocol for ovulation induction to obtain an adequate number of high quality oocytes. Strict quality control and quality assurance is vital in any IVF program to produce viable embryos. Optimizing fertilization has been achieved through ICSI. The technique of embryo transfer has a significant impact on the results. Multiple pregnancy must be avoided, however, in cases of high order multiple pregnancy, the modified technique of multifetal reduction is an option to improve the outcome.

Further research should focus on studying distal tubal opening with or without proximal tubal ligation as compared to salpingectomy in patients with hydrosalpinges before undergoing IVF. For optimizing the technique of ET, future research should focus on investigating different drugs that may decrease uterine contractility during ET. Another area of research is the use of diploid spermatogenic cells to achieve fertilization in non-obstructive azoospermia patients in

whom no spermatozoa could be retrieved. The problem of high order multiple pregnancies should be completely solved and more research should be directed towards improving implantation and single embryo transfer.

SAMENVATTING

In hoofdstuk 1 wordt de geschiedenis van de in vitro fertilisatie (IVF) besproken en worden de resultaten van IVF in de diverse delen van de wereld beschreven. De diverse factoren die het resultaat van IVF beïnvloeden komen aan de orde met nadruk op ovariële stimulatie-protocollen, de ontwikkeling die plaatsvond in de technieken voor het verzamelen van eicellen, de verbetering van de laboratoriumresultaten met name wat betreft de fertilisatie en de ontwikkeling van weefselkweekmedia.

Het doel van dit promotie onderzoek is, zoals besproken in hoofdstuk 2, het optimaliseren van de IVF resultaten door de volgende aspecten extra aandacht te schenken: ten eerste het vooronderzoek van de patiënt en het voorbereiden voor de eerste IVF cyclus, waarbij met name de diagnostiek en de behandeling van hydrosalpingen aan bod komt, het uitvoeren van een dummy embryotransfer, en de diverse factoren die de uitkomst van de embryotransfer zouden kunnen beïnvloeden. Ten tweede de rol van Intra Cytoplasmatische Sperma Injectie (ICSI) bij het pogen de fertilisatieresultaten te verbeteren. Hierbij komt met name het modificeren van de ICSI techniek aan bod, het belang van de afzonderlijke semenparameters, en het gebruik van testiculair en epididymaal verzamelde spermatozoa in het geval van azospermie. Ten derde het bestrijden van een van de negatieve bijwerkingen van IVF, de meervoudige zwangerschap, met behulp van het reduceren van het aantal concepti, en het effect van die reductie op de uitkomst van de zwangerschap.

In hoofdstuk 3 wordt de aanwezigheid van vloeistof in het cavum uteri bestudeerd in relatie tot het voorkomen van hydrosalpingen. Dit lijkt een negatief effect te hebben op de implantatiekansen van het embryo na IVF. De conclusie die op basis van dit, en later in de literatuur gerapporteerd, onderzoek getrokken wordt is dat bij patiënten met hydrosalpingen een salpingectomie moet worden aanbevolen alvorens tot IVF over te gaan.

In hoofdstuk 4 wordt aangetoond dat het uitvoeren van een dummy embryotransfer de IVF resultaten significant verbetert. Het uitvoeren van een dummy embryotransfer stelt de arts in staat de beste katheter voor de transfer op voorhand te kiezen, de diepte van het cavum uteri te bepalen alsmede de hoek waaronder dit zich presenteert, terwijl tevens onvoorziene problemen bij de terugplaatsing kunnen worden opgespoord.

In hoofdstuk 5 wordt additioneel onderzoek beschreven naar de techniek van embryotransfer met behulp van methyleenblauw kleurstof. Het effect van diverse factoren op de expulsie van de kleurstof uit het cavum uteri werd bestudeerd. Er werd gevonden dat de mate van expulsie van de kleurstof significant minder was indien zachte katheters werden gebruikt, in vergelijking met meer rigide katheters, en indien het cervixslijm voor de transfer eerst werd geaspireerd.

In hoofdstuk 6 wordt het effect van de afzonderlijke semenparameters op de uitkomst van ICSI bestudeerd. Fertilisatie en zwangerschapscijfers na ICSI blijken niet te worden beïnvloed door de afzonderlijke semenparameters voorzover er morfologisch normale zaadcellen gebruikt worden voor de Intra Cytoplasmatische Sperma Injectie. Daarnaast wordt aangetoond dat patiënten die tevoren een mislukte fertilisatie bij reguliere IVF hadden, met vrucht gebruik kunnen maken van ICSI om een hernieuwde fertilisatiestoornis te voorkomen.

In hoofdstuk 7 wordt een studie beschreven naar het gebruik van epididymaal en testiculair verzamelde zaadcellen bij ICSI. Het bleek dat ICSI met epididymaal of testiculair zaad in gevallen van obstructieve azospermie een efficiënte methode is om fertilisatie en zwangerschap te bewerkstelligen.

In hoofdstuk 8 wordt een prospectief gerandomiseerde studie beschreven bij "sibling" eicellen om te bestuderen of cytoplasmatische aspiratie nodig is bij het uitvoeren van ICSI. De studie toont aan dat dit niet essentieel is voor eicelactivatie aangezien het de fertilisatiefrequentie niet verhoogt. Wel bestaat het risico dat cytoplasmatische aspiratie de mate van schade aan de eicel

verhoogt. De techniek zonder aspiratie resulteerde in een significant hogere frequentie van embryo's van een goede kwaliteit in vergelijking met de techniek waarbij wel cytoplasma wordt geaspireerd voordat de zaadcel in de eicel wordt geïnjecteerd.

In hoofdstuk 9 wordt een onderzoek beschreven naar de uitkomst van ICSI bij obstructieve en non-obstructieve azospermie. ICSI bij patiënten met een verworven obstructieve azospermie bleek in een significant hogere fertilisatie- en zwangerschapsfrequentie te resulteren dan ICSI bij patiënten met een congenitale obstructieve of non-obstructieve azospermie. Er werd geen verschil gevonden in fertilisatie- en zwangerschapsfrequenties na ICSI wegens obstructieve azospermie tussen epididymale en testiculaire zaadcellen. De resultaten van dit onderzoek laten ook zien dat het fertilisatievermogen van testiculair verzamelde zaadcellen bij non-obstructieve azospermie significant lager is dan bij obstructieve azospermie. Tenslotte werd aangetoond dat zaadcellen konden worden gewonnen uit 67% van de testisbiopsieën bij patiënten met een non-obstructieve azospermie.

In hoofdstuk 10 wordt een gemodificeerde techniek beschreven om het aantal embryo's te reduceren in geval van meerlingzwangerschappen. De gemodificeerde techniek bestaat uit het aspireren van embryonale delen en maakt het gebruik van KCL als cardiotoxische substantie overbodig. De techniek kan bij 7 tot 8 weken zwangerschap via transvaginale ultrageluidsaspiratie van de foetale echo's worden uitgevoerd. De na toepassing van deze techniek resterende tweelingzwangerschappen hadden een uitkomst die vergelijkbaar was met die van "gewone" na IVF ontstane tweelingzwangerschappen. Reductie van meerlingen tot tweelingen kan aldus veilig geschieden en de prognose van de zwangerschap verbeteren.

In hoofdstuk 11 worden de bevindingen van de voorgaande hoofdstukken geïntegreerd met literatuurbevindingen en wordt een overzicht gegeven van alle beschreven ontwikkelingen in het kader van het optimaliseren van de IVF resultaten. Het wordt benadrukt dat IVF een gevoelige en complexe procedure is waarvan het succes van vele factoren afhangt. Het belang

van een goed team wordt benadrukt, evenals het goed voorbereiden van de patiënt op de behandeling. De diagnostiek en behandeling van hydrosalpingen wordt in een breder kader geplaatst, evenals het uitvoeren van dummy embryotransfers in het kader van het kiezen van een optimale terugplaatskatheter. Ook komen in dit hoofdstuk de diverse ovulatie inductie protocollen aan de orde die tot doel hebben eicellen van voldoende hoge kwaliteit te verkrijgen. Quality Control and Quality Assurance zijn essentieel voor een goed IVF programma. ICSI kan de resultaten, met name bij patiënten met fertilisatiestoornissen, verbeteren. De techniek van embryotransfer verdient meer aandacht. Het is van groot belang meervoudige zwangerschappen te voorkomen, echter het reduceren van het aantal embryo's is een efficiënte methode om de uitkomst van zulke zwangerschappen te verbeteren.
Toekomstig onderzoek zal zich moeten concentreren op alternatieve behandelingen voor hydrosalpingen dan salpingectomie. Tevens zal meer aandacht geschonken moeten worden aan het verbeteren van de embryo terugplaatstechniek, o.a. in relatie tot de inherente en de geïnduceerde contractiliteit van de (niet zwangere) uterus. Een ander toekomstig onderzoeksgebied is gelegen in het gebruik van diploïde spermatogene cellen die gebruikt kunnen worden om fertilisatie te bewerkstellingen bij patiënten bij een non-obstructieve azo spermie bij wie op geen enkele wijze zaadcellen kunnen worden gewonnen. Het ultieme doel van de moderne IVF dient het voorkomen van meerlingzwangerschappen te zijn. Het valt te verwachten dat meer onderzoek naar het verbeteren van de implantatiekansen van individuele embryo's zal leiden tot electieve terugplaatsing van één enkel embryo.

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Curriculum Vitae

Ragaa Taha Ahmed Mansour was born on January 1st, 1950 in Kalyobia, Egypt. She graduated from the Faculty of Medicine, Cairo University, in 1973, with honors. This was followed up by completing a three-year residency in Obstetrics and Gynecology at Cairo University (1975 – 1978) during which she earned her master's degree in Ob/Gyn. She then completed her Educational Commission for Foreign Medical Graduates (ECFMG) Certification, in Kansas City (USA), in 1980. She completed a postdoctoral fellowship "in vitro fertilization and embryo transfer" at Ohio State University, USA, 1982. In 1985, she returned back to her home country, and started the first in-vitro fertilization program in Egypt, in March 1986. She has held the position of director of The Egyptian IVF-ET Center since 1986 until the present. In addition, today she is a deputy editor of the Middle East Fertility Society Journal, and a corresponding editor for the American Journal of Obstetrics and Gynecology. She is a member of many reproductive medicine societies in Egypt, Europe and the United States. She is currently the author of 93 scientific publications.

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